



## Neurite outgrowth in response to cerebrospinal fluid derived from Nec-sensitive preterm pigs

Chatterton, Dereck Edward Winston; Sangild, Per Torp

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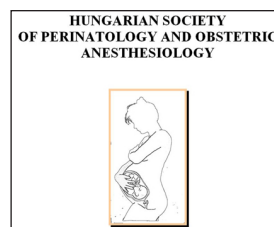
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## POSTER PRESENTATIONS

## ABS 1

### SEPARATE EFFECTS OF LOW PATERNAL AND MATERNAL EDUCATIONAL LEVEL ON RISK OF DEVELOPMENTAL DELAY IN 4-YEAR-OLD BOYS AND GIRLS

S. de Jong<sup>1</sup>, M.R. Potijk<sup>2</sup>, A.E. den Heijer<sup>1</sup>, S.A. Reijneveld<sup>2</sup>, A.F. Bos<sup>1</sup>, J.M. Kerstjens<sup>1</sup>

<sup>1</sup>Division of Neonatology, Department of Paediatrics, University Medical Center Groningen, Groningen, the Netherlands

<sup>2</sup>Department of Health Sciences, Community and Occupational Medicine, University Medical Center Groningen, Groningen, the Netherlands

## INTRODUCTION

The effects of maternal and paternal educational level on children's development may differ per gender. Some previous findings suggested stronger effects of mothers on daughters and of fathers on sons, respectively. However, as far as we know, the gender-effects of maternal and paternal educational level on risk of overall developmental delay have not been assessed before. Therefore, our objective was to examine whether the association between both maternal and paternal educational level and risk of developmental delay differs by the offspring's gender.

## PATIENTS AND METHODS

We used data from a community-based cohort (LOLLIPOP), including 1,438 preterm- and 544 term-born children born in 2002/2003. Ages and Stages Questionnaires (ASQ) total scores and domain scores > -2 SD below the mean indicated developmental delay. We computed odds ratios (OR) and 95% confidence intervals (CI) to assess crude effects of low and intermediate compared to high paternal and maternal educational level on the risk of developmental delay. Secondly, we adjusted for child factors (SGA, gender,

multiples, gestational-age group, age at ASQ administration) and parental factors (parity, ethnicity and educational level of the other parent). Finally, in two separate regression models, we added the interaction between the terms "paternal educational level" and "gender", and "maternal educational level" and "gender".

## RESULTS

Regarding ASQ total and domain scores, no significant interactions were found between paternal educational level and gender (all  $p > .05$ ). In contrast, significant interactions between low maternal educational level and gender were found for the ASQ total score, personal-social skills and problem-solving ( $p = 0.013$ ,  $p = 0.042$  and  $p = 0.036$  respectively). In analyses stratified by gender, the risk of overall developmental delay was nearly fourfold in daughters of low educated mothers compared to daughters of high educated mothers (OR 3.83, CI 1.23-11.9). We found no difference in rates of developmental delay between sons of low educated mothers and sons of high educated mothers (OR 1.13, CI 0.57-2.24). Regarding the ASQ domains, low maternal education increased the risk of delay in personal-social skills in girls (OR 3.72, CI 1.10-12.6) but not in boys (OR 1.29, CI 0.59-2.81).

## CONCLUSIONS

Low paternal educational level increased the risk of developmental delay in both sons and daughters. Low maternal educational level increased the risk of developmental delay in daughters only. These findings suggest that the effect of low maternal educational level on developmental delay differs per gender. If other studies would confirm our findings, this may lead to better insights in the effects of parental education on children's development.

## ABS 2

### THE ASSOCIATION BETWEEN PATERNAL EDUCATIONAL LEVEL AND DEVELOPMENTAL DELAY IN PRETERM AND TERM-BORN CHILDREN AT AGE 4

S. de Jong<sup>1</sup>, J.M. Kerstjens<sup>1</sup>, A.E. den Heijer<sup>1</sup>, A.F. Bos<sup>1</sup>, S.A. Reijneveld<sup>2</sup>, M.R. Potijk<sup>2</sup>

<sup>1</sup>Division of Neonatology, Department of Paediatrics, University Medical Center Groningen, Groningen, the Netherlands

<sup>2</sup>Department of Health Sciences, Community and Occupational Medicine, University Medical Center Groningen, Groningen, the Netherlands

## INTRODUCTION

It is well known that maternal socioeconomic status (SES), e.g. maternal educational and occupational level, influences the risk of neurodevelopmental problems in offspring. In contrast, little is known about the effect of paternal SES on neurodevelopmental problems. Furthermore, it is unknown whether the effect of paternal SES on development differs between preterm-born and term-born children. Therefore, our objectives were to determine the association between paternal educational level and risk of developmental delay in four-year-old children, adjusting for maternal and child indicators, and to determine whether the effect of paternal education differed by gestational age.

## PATIENTS AND METHODS

We used data from a community-based cohort (LOLLIPOP), including 1,438 preterm- and 544 term-born children born in 2002/2003. Ages and Stages Questionnaires (ASQ) total scores and domain scores  $> -2$  SD below the mean indicated developmental delay. First, we computed crude odds ratios (ORs) and 95% confidence intervals (CIs) for the association between low and intermediate paternal educational level and risk of developmental delay, compared to high paternal educational level. Second, we adjusted for child (SGA, gender, multiple gestation, gestational age group and age at ASQ administration) and maternal factors (parity, ethnicity and maternal educational level). Third, we added the interaction between the terms “paternal educational level” and “gestational age” to the regression model.

## RESULTS

Low paternal educational level significantly increased the overall risk of developmental delay (ASQ total score) (OR 2.85, CI 1.80-4.51). This effect remained significant after full adjustment for child and maternal risk factors (OR 2.42, CI 1.39-4.20). Regarding ASQ domains, low paternal educational level significantly increased the risk of delay in fine motor, problem-solving and personal-social skills after adjustment for child and maternal risk factors (OR 2.30, CI 1.34-3.96; OR 3.32, CI 1.72-6.42; and OR 1.95, CI 1.04-3.67, respectively), but not the risk of delay in gross motor function and communication skills (OR 1.34, CI .80-2.26 and OR 1.58, CI .98-2.55, respectively). No significant interaction between paternal educational level and gestational age was found ( $p > .05$ ).

## CONCLUSIONS

Low paternal educational level is associated with an increased risk of developmental delay at age 4,

also after adjustment for maternal factors. This association did not differ by gestational age. Regarding developmental domains, low paternal educational level significantly increased the risk of delay in fine motor, problem-solving and personal-social skills. Paternal SES should therefore be accounted for in neonatal follow-up studies.

# Early Developmental Care

## ABS 3

### NEUROPROTECTION BY NEURONAL OVER-EXPRESSION OF THE SMALL GTPase-Ras IN HYPEROXIA-INDUCED NEONATAL BRAIN INJURY

M. Serdar, K. Kempe, J. Herz, R. Herrmann, B.S. Reinboth, R. Heumann, A. Ehrkamp, U. Felderhoff-Mueser, I. Bendix

*Department of Pediatrics I/Neonatology, University Hospital Essen, Essen, Germany*

## INTRODUCTION

Premature born infants are highly susceptible to different environmental factors, such as high oxygen concentration. As demonstrated previously, hyperoxia induces perinatal brain injury affecting white and gray matter structures that are ameliorated by a neuronal overexpression of Ras. However, the underlying molecular and cellular mechanisms, particularly the contribution of neurons and/or oligodendrocyte subsets to the described phenotype, remain elusive. Therefore, we were interested whether neuronal overexpression of Ras affects hyperoxia-mediated white matter injury.

## PATIENTS AND METHODS

synRas mice, characterised by a neuronal Ras overexpression, or wildtype littermates were used in all experiments. Briefly, six-day-old mice were kept under hyperoxic (80% O<sub>2</sub>) or room air (21% O<sub>2</sub>) conditions for 24 h. Cell death of oligodendrocytes was analysed by immunohistochemistry and western blotting at P7 with different neural markers like Olig2, NG2, PDGFR $\alpha$ , NeuN, cCaspase3. The differentiation capacity of oligodendrocytes was assessed by quantification of myelin basic protein (MBP) expression at P11. Long term changes of myelin structures were evaluated via transmission



electron microscopy (TEM) analysing myelin thickness and integrity at P40.

## RESULTS

Western blot analysis of active Caspase-3 demonstrates a significant upregulation in control mice exposed to hyperoxia. However, hyperoxic synRas mice do not show a marked alteration of cleaved Caspase-3 protein expression. Furthermore, we detected a protective effect of neuronal Ras overexpression on survival of oligodendrocytes after hyperoxia. Whether this effect is due to an overall decrease of neuronal cell death and which molecular targets are involved in this protection remains to be investigated. Interestingly, at P11 we detected an amelioration of disturbed myelination in synRas mice compared to hypomyelinated littermates exposed to hyperoxia. Accordingly, we detected long-lasting structural alterations of myelins sheaths of hyperoxic wild type mice compared to normoxic wild type mice whereas synRas mice do not reveal marked alterations.

## CONCLUSIONS

Neuronal overexpression of Ras protects from hyperoxia-mediated cell death accompanied by an increased oligodendrocyte survival, myelination and long-lasting improvement of myelin structures. Further work is required to dissect the underlying molecular mechanisms in order to understand the complex communication network between neurons and oligodendrocytes during brain maturation under pathological but also physiological conditions.

## ABS 4

### REFERENCE RANGES FOR CEREBRAL TISSUE OXYGEN INDEX (cTOI) IN NEONATES DURING IMMEDIATE NEONATAL TRANSITION AFTER BIRTH

N. Baik, B. Urlsberger, B. Schwabegger, G. Schmörlzer, A. Avian, L. Mileder, G. Pichler

<sup>1</sup>Division of Neonatology, Department of Pediatrics, Medical University of Graz, Graz, Austria

<sup>2</sup>Research Unit for Neonatal Micro- and Macrocirculation Department of Pediatrics, Medical University, Graz, Austria

## INTRODUCTION

Non-invasive monitoring of the brain with near-infrared-spectroscopy (NIRS) during immediate transition after birth is of growing interest.

## OBJECTIVE

To define reference ranges and centile charts for regional cerebral tissue oxygenation index (cTOI) measured with NIRO® 200NX (Hamamatsu, Japan) and cerebral-fractional-tissue-oxygen-extraction (cFTOE) during the first 15 minutes after birth in pre-term and term neonates without any medical support.

## PATIENTS AND METHODS

Methods: cTOI was measured with NIRO® 200NX during the first 15 minutes after delivery via caesarean section in preterm and term infants. The NIRS-sensor was placed on the right forehead. Peripheral arterial oxygen saturation (SpO<sub>2</sub>) and heart rate were continuously measured by pulse oximetry. cFTOE was calculated out of cTOI and SpO<sub>2</sub>. Neonates with requirement for any medical support were excluded.

## RESULTS

A total of 230 neonates were enrolled: 82 neonates had to be excluded due to respiratory support. 148 neonates (140 term neonates/8 preterm) were included and data were used to define reference ranges and centile charts.

50<sup>th</sup> centile (10<sup>th</sup>-90<sup>th</sup> centiles) of cTOI was 55% (38-75) at 2 min, 65% (50-78) at 5 min, 74% (61-85) at 10 min, and 74% (61-84) at 15 min after birth.

50<sup>th</sup> centile (10<sup>th</sup>-90<sup>th</sup> centiles) of cFTOE was 0.24 (0.11-0.44) at 2 min, 0.20 (0.10-0.35) at 5 min, 0.21 (0.09-0.35) at 10 min, and 0.24 (0.13-0.37) at 15 min after birth.

## CONCLUSIONS

The present observational study adds the reference ranges and centile charts of cTOI measured with NIRO® 200NX and cFTOE calculated out of cTOI and SpO<sub>2</sub> in neonates during the immediate neonatal transition. Centiles for each instrument will be necessary for future clinical application, since difference between cTOI and crSO<sub>2</sub> changes with increasing regional oxygenation.

## ABS 5

### PSYCHOTROPIC DRUG USE DURING PREGNANCY: IS PROFESSIONAL OBSERVATION OF MOTHER AND INFANT NEEDED POST PARTUM?

F. de Jong<sup>1</sup>, F. Scheele<sup>2</sup>, K. Dolman<sup>3</sup>, A. Honig<sup>1</sup>

<sup>1</sup>Department of Psychiatry, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands

<sup>2</sup>Department of Gynaecology, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands

<sup>3</sup>Department of Paediatrics, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands

## INTRODUCTION

Psychiatric disorders and use of psychotropic drugs during pregnancy may lead to complications post partum, such as Poor Neonatal Adaptation (PNA) and psychiatric decompensation after delivery. Evidence based recommendations regarding the duration of post partum observation of mothers who used psychotropic drugs during pregnancy and their infants are lacking. This study aimed to provide these evidence based recommendations by analyzing the type, number and time to performed medical interventions during the first days post partum in this patient group.

## PATIENTS AND METHODS

This observational study was performed in the Sint Lucas Andreas Hospital in Amsterdam. We included mother-infant dyads who were admitted to the maternity ward between January 2007 and December 2012 for observation of possible complications caused by exposure of psychotropic drugs during pregnancy. We analyzed the type, number and time to medical interventions which had to be the result of complications due to the maternal psychiatric disorder or psychotropic drug use during pregnancy. Interventions were

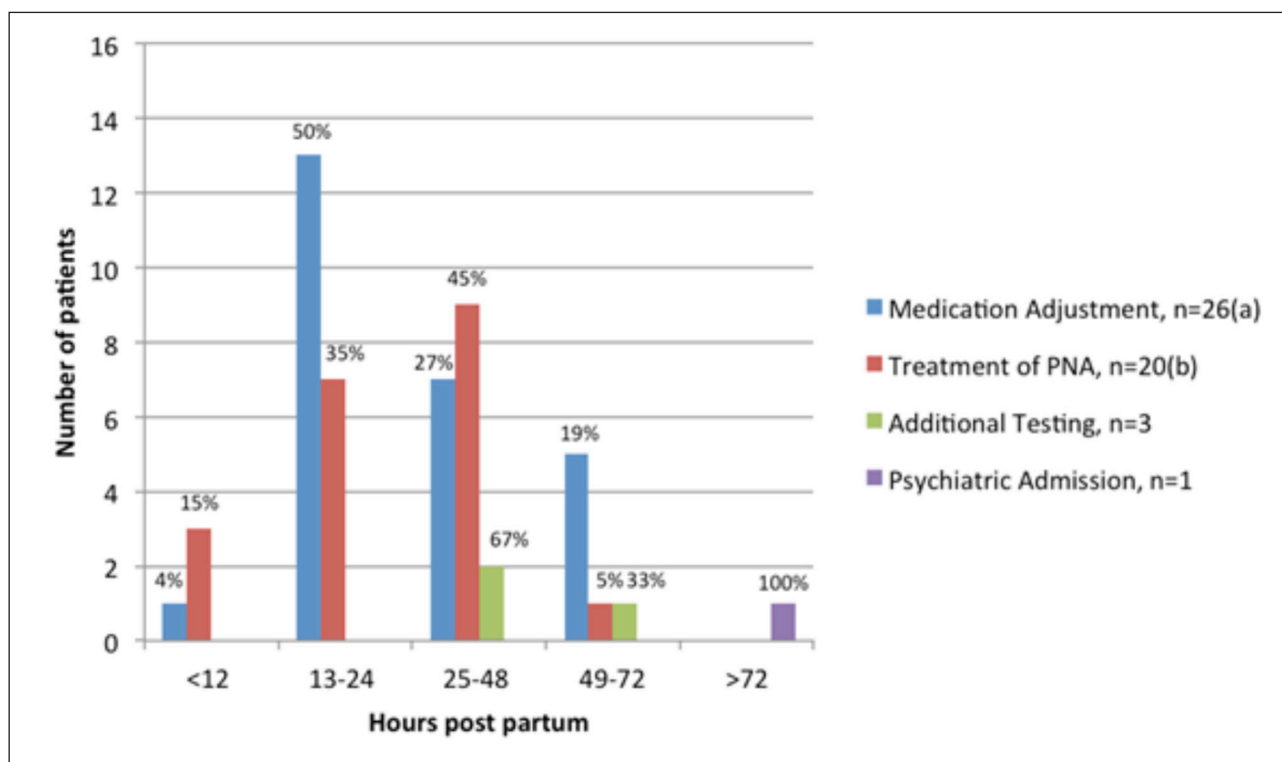
defined as: 1. adjustment of psychotropic drugs, 2. admission to the psychiatric department, 3. additional investigations due to elevated Finnegan scores whereby the final diagnose was PNA, 4. treatment of PNA and 5. consultation of an external organization for additional care.

## RESULTS

Of all 358 mother-infant dyads, 182 (50.8%) had an additional indication for clinical observation, such as perinatal infection, and where therefore excluded. Of the remaining 176 mother-infant dyads, in 71 (40%) one or more interventions were performed. In 84% of the mother-infant dyads whereby an intervention was performed, the final intervention was performed within 48 hours. The most prevalent interventions were adjustment of psychotropic drugs and treatment of PNA (**Fig. 1**).

## CONCLUSIONS

The high prevalence and type of medical interventions warrants professional observation of all mothers who used psychotropic drugs during pregnancy together with their infants. In the absence of specialized home care, clinical observation is indicated whereby an observation period of 48 hours seems sufficient for most mother-infant dyads. Studies examining risk factors of interventions and prolonged admission would have additional value.



**Figure 1 (ABS 5).** Most prevalent interventions in the first days post partum.

## ABS 6

**N-ACETYLCYSTEINE AMIDE (NACA) REDUCES CELL DEATH AFTER EXPOSURE TO OXIDATIVE STRESS IN A PORCINE EPITHELIAL-LIKE EMBRYONIC EFN-R KIDNEY CELL LINE**

T. Benterud<sup>1</sup>, S. Manueldas<sup>1</sup>, L. Pankratov<sup>1</sup>, R. Solberg<sup>1</sup>, A. Nordgren<sup>2</sup>, O.D. Saugstad<sup>1</sup>, L.O. Baumbusch<sup>1</sup>

<sup>1</sup>Department of Pediatric Research, Oslo, Norway

<sup>2</sup>Karolinska University Hospital, Stockholm, Sweden

## INTRODUCTION

Reactive oxygen species (ROS) are important in different processes of the organism, such as cell-signaling. However, during oxidative stress, too much ROS is produced, which may have detrimental effects on different structures of the cells. ROS play a role as a mediator of apoptosis and may induce damage of the DNA. One important type of ROS is Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) which is quite damaging to DNA. We hypothesized that NACA (a new antioxidant and scavenger) could have protective effect on cells exposed to H<sub>2</sub>O<sub>2</sub>.

## PATIENTS AND METHODS

EFN-R (Friedrich-Löffler Institut, Greifswald), used as model system for stress experiments. The cells were starved for 24 h and the confluent cells were treated with H<sub>2</sub>O<sub>2</sub> for various incubation time and concentrations. In experiment I, 5 groups of cells were treated with H<sub>2</sub>O<sub>2</sub> or/and NACA. Group 1 (control group) Without H<sub>2</sub>O<sub>2</sub> or NACA. Group 2 (control H<sub>2</sub>O<sub>2</sub>). Group 3 (control NACA). Group 4 (pre-treatment). Cells were first treated 1 hour with 750 µM NACA, followed by 100 µM H<sub>2</sub>O<sub>2</sub>. Group 5 (post-treatment) Cells were treated with 100 µM H<sub>2</sub>O<sub>2</sub> in 1 h before 750 µM NACA was added. The cell viability was measured by the MTT assay. qRT-PCR was used for measuring the gene expression of Caspase3 and Bax.

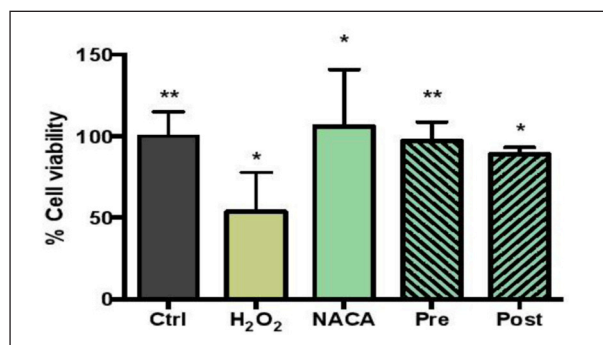
Random Mutation Capture (RMC) method, was used to quantify damage of mitochondrial (mtDNA) and NuclearDNA (ntDNA).

## RESULTS

The peroxide control group (2) had a significantly lower cell viability compared to the control group without any treatment,  $0.45 \pm 0.21$  vs.  $0.83 \pm 0.13$  mutational ratio ( $p < 0.001$ ).

EFN-R cells exposed to pre- (group 4) or post-treatment (group 5) of NACA, both exhibited significantly increased viability ( $0.81 \pm 0.10$ ,  $p <$

$0.01$  and  $0.73 \pm 0.05$ ,  $p < 0.05$ , respectively), without significant difference between pre- vs. post-treatment (Fig. 1). Similar observation were observed for ntDNA. Gene expression of Caspase 3 and Bax were significantly decreased for cells post-treated with NACA in comparison to H<sub>2</sub>O<sub>2</sub> exposure cells.



**Figure 1 (ABS 6).** Effect of NACA on cells exposed to different concentrations of H<sub>2</sub>O<sub>2</sub>. The cells were divided into: control, H<sub>2</sub>O<sub>2</sub>, only NACA, pre-treatment or post-treatment groups. In the pre-treatment group, cells were initially exposed to 750 µM NACA for 30 minutes, and thereafter treated for H<sub>2</sub>O<sub>2</sub> for 1 hour. In the post-treatment group, the cells were treated for H<sub>2</sub>O<sub>2</sub> for 30 minutes before NACA-treatment. Cells were incubated for 1 hour and the viability was assessed by MTT assay. Value represents means  $\pm$  SD, and  $n = 8$ . Statistically different values of \* $p < 0.05$  and \*\* $p < 0.01$  were calculated with t-test compared to H<sub>2</sub>O<sub>2</sub> control.

## CONCLUSIONS

In our study, we revealed that NACA, after exposure of H<sub>2</sub>O<sub>2</sub>, had a protective effect regarding DNA damage. NACA may have an inhibiting effect on the gene expression of the pro-apoptotic components Bax and Caspase 3, as well as increasing the cell viability after exposure of H<sub>2</sub>O<sub>2</sub>. Taking together, our results suggest that NACA may play an important role in reducing pathological complication in several disorders involving oxidative stress reactions.

## ABS 7

**MELATONIN-INDUCED IMMUNE CELL RESPONSES IN INFANTS WITH NEONATAL ENCEPHALOPATHY PRE AND POST THERAPEUTIC HYPOTHERMIA TREATMENT**

S. Aslam<sup>1</sup>, V. McEneaney<sup>2</sup>, R.W.G. Watson<sup>3</sup>, A. O'Neill<sup>3</sup>, E.J. Molloy<sup>2,4,5</sup>

<sup>1</sup>Neonatology, National Maternity Hospital, Holles Street, Dublin, Ireland

<sup>2</sup>Academic Paediatrics, Trinity College Dublin, National Children's Hospital, Tallaght, Dublin, Ireland

<sup>3</sup>Conway Institute for Biomolecular & Biomedical Science, University College Dublin, Ireland

<sup>4</sup>Neonatology, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland

<sup>5</sup>Paediatrics, Coombe Women's and Infant's University Hospital, Dublin, Ireland

## INTRODUCTION

Infection and inflammation are associated with Neonatal Encephalopathy (NE) and increase the risk of neurological sequelae. Melatonin is a potent immunomodulator and antioxidant and may alter the systemic inflammatory response in NE.

## AIM

To investigate the *in vitro* effect of melatonin treatment on neutrophils and monocytes activation in infants with NE pre and post therapeutic hypothermia (TH).

## PATIENTS AND METHODS

Infants with NE  $\geq$  Sarnat grade II ( $n = 15$ ) and neonatal controls ( $n = 10$ ) were recruited. Whole blood samples were taken on Day 1, 3 and 7 from infants with NE receiving therapeutic hypothermia and on day 1 in neonatal controls. Whole blood samples were treated with Lipopolysaccharide (LPS 1  $\mu\text{g}/\text{ml}$ ), melatonin (10-3 M) and LPS and melatonin. Reactive oxygen intermediates (ROI) play a role in bacterial killing. Cluster differentiation (CD11b, B2 integrin) is a surface receptor that aids the adherence of neutrophils to the endothelial cell wall, facilitating their migration to the site of injury. Toll like receptor-4 (TLR4) is the major recognition receptor for endotoxin lipopolysaccharide (LPS). Flow cytometry was performed to assess TLR4, CD11b and ROI expression in monocytes and neutrophils.

## RESULTS

Infants with NE  $\geq$  Sarnat grade II ( $n = 15$ ) and neonatal controls ( $n = 10$ ) were recruited. Monocyte LPS-induced ROI production was significantly increased ( $p = 0.03$ ) in NE vs. controls on day 1 of life. Following TH, neutrophil LPS-induced CD11b upregulation was significantly decreased by melatonin *in vitro* in neonates with NE on day 1 and 7 of life. There was no difference in baseline and LPS-induced TLR4 expression in either NE or controls.

## CONCLUSIONS

Melatonin decreases the expression of neutrophil CD11b in infants with NE pre and post therapeutic hypothermia therapy. Reduced CD11b expression in response to melatonin may ameliorate the augmented systemic inflammatory response seen in infants with NE.

## ABS 8

### BLOOD PRESSURE DURING THE IMMEDIATE NEONATES TRANSITION: IS THE MIDDLE ARTERY PRESSURE (MAP) RELEVANT FOR THE REGIONAL CEREBRAL OXYGENATION ( $\text{crSO}_2$ )?

N. Baik, B. Urlesberger, B. Schwabegger, G. Schmölzer, A. Avian, G. Pichler

<sup>1</sup>Division of Neonatology, Department of Pediatrics, Medical University of Graz, Graz, Austria

<sup>2</sup>Research Unit for Neonatal Micro- and Macrocirculation Department of Pediatrics, Medical University, Graz, Austria

## OBJECTIVE

The objective of this study was to investigate a potential influence of mean arterial pressure (MAP) on the regional cerebral oxygenation ( $\text{crSO}_2$ ) in preterm and term infants during the immediate neonatal transition.

## PATIENTS AND METHODS

In this prospective observational study preterm and term infants were included. The regional cerebral oxygenation ( $\text{crSO}_2$ ) was measured by near-infrared spectroscopy (NIRS) with the INVOS 5100C during the immediate neonatal transition (15 minutes immediately after birth). The NIRS sensor was applied to the left frontal forehead. Furthermore, a pulse oximeter was applied to monitor arterial oxygen saturation ( $\text{SpO}_2$ ) and the heart rate (HR). In the 15<sup>th</sup> minute after birth the blood pressure was measured non-invasively on the left upper arm. Cerebral fraction tissue oxygen extraction (cFTOE) was calculated from  $\text{SpO}_2$  and  $\text{crSO}_2$ . To investigate the association between cFTOE and MAP, a correlation analysis was performed.

## RESULTS

A total of 462 preterm and full-term infants (186/276) were included. The mean gestational age was  $31.0 \pm 3.5$  weeks for preterm infants and  $38.9 \pm 0.8$  weeks at full-term infants. The mean birth weight was 1,591  $\pm$  630 g in preterm infants, 3,331  $\pm$  461 g in term infants. The correlation analysis showed no statistically significant association between MAP and cFTOE in term infants, in contrast, there was a statistically significant negative correlation in preterm infants between MAP and cFTOE ( $p = 0.02$ ).

## CONCLUSIONS

MAP has a significant impact on cerebral oxygenation in preterm infants. The monitoring of the MAP already during the immediate neonatal



transition in preterm infants may be relevant in clinical practice in order to influence the cerebral oxygenation positively with an eventual therapy of the MAP.

## ABS 9

### BETWEEN 1993 AND 2012: EVOLUTION OF CEREBRAL PALSY IN PRETERM CHILDREN

M. Bickle Graz, J. Schneider, A.C. Truttmann

*Neonatology, Department of Pediatrics, University Hospital and University of Lausanne, Lausanne, Switzerland*

#### INTRODUCTION

The incidence of cerebral palsy (CP) in preterm children is correlated to gestational age, and has been reported to decrease. The objective of this study is to compare the incidence and risk factors for CP, as well as the subtype and severity of CP between 2 ten-year periods.

#### PATIENTS AND METHODS

Retrospective analysis of a cohort of very preterm infants hospitalized in our level III NICU and born from 1993-2002 (n = 873) and 2003-2012 (n = 987). There was no change in mean birthweight, but mortality decreased from 14.4% to 10.3% (p = 0.07). Follow-up was offered to the 1,632 (87.7%) survivors and performed in 88% of patients. Cerebral palsy was diagnosed at the 18 months visit and was confirmed at a later appointment. In the first years children were evaluated with the Griffiths Mental Development Scales, and with the Bayley Scales, 2<sup>nd</sup> edition later on. Severity was assessed with cognitive as well as sensorial function, and with the Gross Motor Function Classification system (GMFCS).

#### RESULTS

1,446 children were evaluated, 68 presented with cerebral palsy (4.7%), among them 32% with spastic quadriplegic, 33% spastic diplegic, 32% spastic hemiplegic and 3% ataxic subtypes. Rate of CP was 3.7% of survivors in period 1, 4.75% in period 2 (p = 0.156), with no difference in birthweight (p = 0.629) or gestational age (p = 0.361) between the 2 periods. Assessment with the GMFCS showed that 66% in the first period and 80% in the second period had scores 1-2, (p = 0.158), with age at walking significantly lower in period 2 (21.5 months) than period 1 (30.0 months), (p = 0.03). Preliminary results show a diminishing severity of CP and comorbidities across the years.

## CONCLUSIONS

Despite improvements in neonatal care and increased survival, CP incidence has slightly increased, but its quality and severity have changed, and need to be discussed.

## ABS 10

### N-ACETYL CYSTEINE AMIDE (NACA) MAY HAVE NEUROPROTECTIVE PROPERTIES AFTER PERINATAL ASPHYXIA

T. Benterud<sup>1</sup>, L. Pankratov<sup>1</sup>, R. Solberg<sup>1</sup>, A. Nordgren<sup>2</sup>, L.O. Baumbusch<sup>1</sup>, O.D. Saugstad<sup>1</sup>

<sup>1</sup>Department of Pediatric Research, Oslo, Norway

<sup>2</sup>Karolinska University Hospital, Stockholm, Sweden

#### INTRODUCTION

The WHO estimated in 2010 that more than 700,000 children die yearly because of complications after perinatal hypoxia. Even though the majority of the children survive, many of them will suffer from reduced physical or mental skills. Medication is required to reduce the neurological damages after asphyxia. Our group is the first one investigating the possible neuroprotective abilities of NACA, a drug with potential anti-inflammatory and anti-oxidative properties, using a pig model of perinatal asphyxia.

#### PATIENTS AND METHODS

Newborn pigs (n = 54), age 12-36 h, were exposed to global hypoxia until BE was either -15 or -20 mmol/l (moderate/severe asphyxia with NACA or saline) (4 groups with n = 12 and a control group with n = 6). and invasive blood pressure and ECG were measured continuously. The pigs were observed for 9.5 hours before tissue sampling from prefrontal cortex and cerebellum.

#### Laboratory Methods

Gene expression was examined by ELISA for cytokines and by qRT-PCR. Random Mutation Capture (RMC) was applied to investigate the mutation rate of mitochondrial DNA (mtDNA). *In-situ* zymography was used for measuring the net gelatinolytic activity in the nucleus of Purkinje cells. Caspase 3 and p-p65, the active form of NF-κB, were estimated by Western blots.

#### RESULTS

NACA significantly reduced the levels of protein concentration in cerebral cortex for the pigs exposed to severe hypoxia (p < 0.05 for both proteins), as could be seen for IL-1b and p-p65 (the active form of NF-κB). Dividing the levels of TNF-α in plasma



30 minutes after reoxygenation with the levels at end hypoxia, we observed a significantly lower ratio for pigs exposed to NACA ( $p < 0.02$ ). mtDNA mutation ratios in cerebellum were significantly lower for the pigs exposed to NACA after hypoxia, than for the saline group ( $p < 0.05$ ) (**Fig. 1**). Multiplying the intensity with the size of the nucleus of the Purkinje cells in cerebellum using *in-situ* zymography, there was a significant difference between the two intervention groups ( $p < 0.05$ ). In addition, gene

expression for TNF- $\alpha$  was significantly reduced for pigs exposed to moderate hypoxia compared with the control group ( $p < 0.05$ ).

#### CONCLUSIONS

The reduced Il-1b and p-p65 protein expression and TNF- $\alpha$  gene expression for the pigs receiving NACA after hypoxia, could indicate an anti-inflammatory effect of NACA. The reduction in gelatinolytic activity in the nucleus of Purkinje cells and the lower mtDNA mutation ratio in cerebellum after NACA treatment suggest neuroprotective abilities of NACA when given after perinatal asphyxia.

## Neonatal Brain Injury and Neuroprotection

ABS 11

### BRAIN INTERSTITIAL PH CHANGES DURING THE SUBACUTE PHASE IN A NEWBORN PIGLET PERINATAL ASPHYXIA MODEL

J. Németh, G. Remzsó, V. Tóth-Szűki, F. Domoki

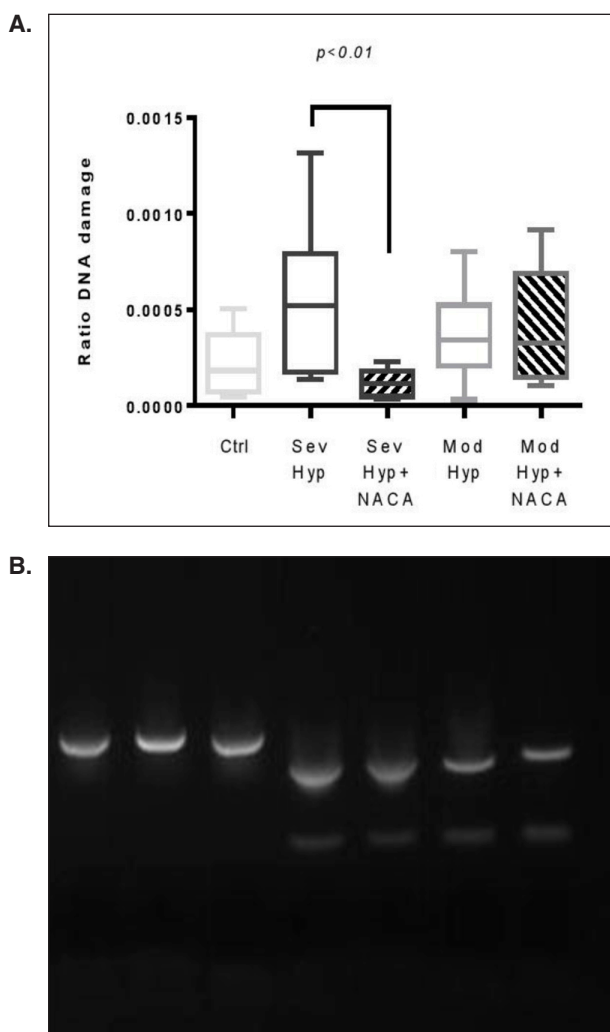
Department of Physiology, University of Szeged, School of Medicine, Szeged, Hungary

#### INTRODUCTION

Perinatal asphyxia (PA) is a major contributor to neonatal mortality and hypoxic-ischemic encephalopathy (HIE). The exact molecular background of HIE is still being investigated, and brain interstitial pH derangements appear to play a critical role in neuronal survival. The newborn piglet is an established large animal model to test the effects of experimental asphyxia. The purpose of the study was to describe postasphyxial brain interstitial (IS) pH changes in the piglet during the first day of survival period as progressive brain alkalosis was reported during this period in a rat PA model previously.

#### PATIENTS AND METHODS

Anesthetized (thiopental, morphine, midazolam), tracheotomized, artificially ventilated, instrumented (femoral vein and carotid artery catheters), and monitored (arterial blood pressure, heart rate, arterial pH, blood gases, lactate levels, O<sub>2</sub> saturation, rectal temperature, EEG) newborn pigs ( $n = 13$ ) were asphyxiated by switching medical-air (21% O<sub>2</sub>, balanced N<sub>2</sub>) ventilation to a hypoxic-



**Figure 1 (ABS 10).** DNA damage in cerebellum. **A.** When comparing the ratio of mutation of DNA between the different groups, there was a significantly lower ratio of mutation for pigs exposed to NACA after severe hypoxia than for the pigs receiving Saline ( $1.1 \times 10^{-4}$ ,  $SD \pm 0.6 \times 10^{-4}$  vs.  $5 \times 10^{-5}$ ,  $SD \pm 3.6 \times 10^{-5}$ ) ( $p < 0.01$ ). **B.** Enzyme digest with EFN-R cells and cerebellum of pigs with their representative enzyme in mtDNA electrophoresed on 3% agarose gel. Enzyme digest with TaqI with three different PCR products containing EFN-R cells and cerebellum. The first three fragments are 12S control gene and the last four fragments are with 12S target gene. The product size in 12S control is 228 bp and for cleavages with TaqI, the product size are 153 bp and 70 bp.

hypercapnic (6% O<sub>2</sub>, 20% CO<sub>2</sub>, balanced N<sub>2</sub>) gas mixture and simultaneously reducing the initial 30 l/min respiratory rate to 15 l/min for 20 minutes. Cortical IS pH was recorded with a proton selective microelectrode through a craniotomy at selected time points.

## RESULTS

Asphyxia elicited severe arterial hypoxemia (PaO<sub>2</sub>: from 65 ± 14 mmHg to 23 ± 5 mmHg; mean ± SD), hypercapnia (PaCO<sub>2</sub>: from 37 ± 12 mmHg to 160 ± 23 mmHg) and acidosis (pH: from 7.53 ± 0.11 to 6.79 ± 0.07); plasma lactate level raised (from 1.6 ± 1 mmol/l to 10.3 ± 3 mmol/l) meanwhile the baseline cortical IS pH (pH: 7.09 ± 0.02) fell also significantly (pH: 5.84 ± 0.32) (**Fig. 1**). Postasphyxial reventilation with air corrected blood gas values within hours and cortical IS pH was also restored during the recovery phase. Interestingly, only 1 animal showed marked alkalosis (maximal pH: 7.84 at 2<sup>nd</sup> hour of survival) during the early reventilation that also returned to baseline later. Vital parameters (core temperature, saturation, blood pressure and blood gas values) were all maintained in the respective physiologic ranges during the 24 hour survival period.

## CONCLUSIONS

The newborn pig is an excellent model to study PA/HIE pathophysiology. The asphyxia-induced drop in pH were quickly restored on both sides of the blood-brain-barrier, and no sign (except in one case) of severe alkalosis was observed described in rat pups. The cause of the obvious discrepancy is unknown, and requires further investigations.

## ABS 12

### NEUROPROTECTIVE EFFECT OF REMIFENTANIL ON EXCITOTOXIC-INDUCED BRAIN DAMAGE IN NEONATAL MICE

C. Chollat<sup>1,2</sup>, F. Tourrel<sup>1,3</sup>, M. Lecointre<sup>1</sup>, Y. Ramdani<sup>1</sup>, S. Marret<sup>1,2</sup>, B. Gonzalez<sup>1</sup>, S. Jégou<sup>1</sup>

<sup>1</sup>Inserm/Region Team ERI 28 Microvascular Endothelium and Neonate Brain Lesions, University of Rouen, Rouen, France

<sup>2</sup>Neonatal Medicine and Intensive Care Dpt, Rouen University Hospital, Rouen, France

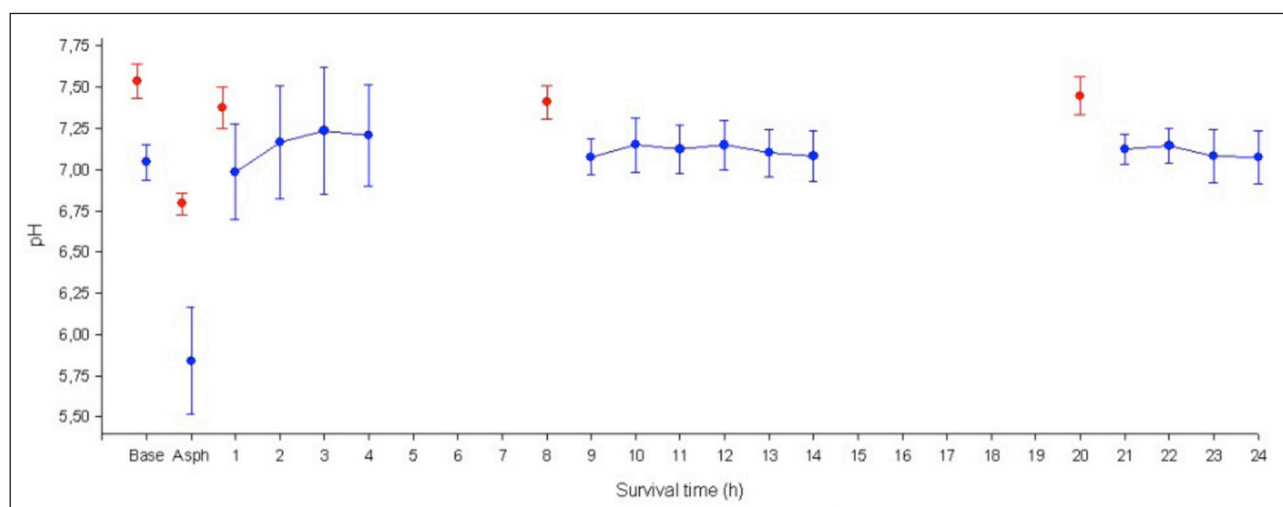
<sup>3</sup>Département of Anesthetics and Intensive Care, Rouen University Hospital, Rouen, France

## INTRODUCTION

Neuroprotection of premature newborns is a public health issue. The goal is, ultimately, to limit motor and cognitive impairments in the neonatal period. We have already shown that the morphinic remifentanyl (Rf), used during caesarian delivery and in neonatal intensive care could be an interesting molecule to this end. Using an *ex vivo* model of brain slices from postnatal day 2 mice (P2), we previously showed that Rf exerts an anti-apoptotic activity (Tourrel et al., Anesth Anal 2014). Considering these initial results, a model of neonatal brain injury by intracortical ibotenate injection was used to evaluate the *in vivo* effects of Rf treatment.

## PATIENTS AND METHODS

3 groups of P2 mice were used: the Rf and NaCl groups received 3 ip injections of Rf (500 µg/kg over a 10-min period) or saline, respectively. Just



**Figure 1 (ABS 11).** Arterial blood and cortical interstitial pH during the experiment. Intermittently measured arterial blood (red scatter) and cortical interstitial (blue scatter) pH values at baseline (Base), at the end of asphyxia (Asph) and during the survival period (1-24 hours). Data expressed as means ± SD.

after the last injection, ic injection of ibotenate (Ibo, 10 µg) was performed. A third group was composed of untreated mice. *In situ* labeling of cortical caspase activity was determined 5 hours after Ibo injection. The lesion size was assessed 5 days after the injection of Ibo. Finally, behavioral tests were done later between 3 and 12 days of life: righting and grasping reflex, negative geotaxis.

## RESULTS

Cortical caspase substrate consumption was significantly lower in Rf group (n = 11) vs. NaCl group (n = 7) (62.45 vs. 100%). The size of the Ibo-induced lesion was significantly reduced in the Rf group (n = 32) vs. NaCl group (n = 32) (226 ± 47 vs. 699 ± 101 µm). Behavioral results showed that in the negative geotaxis test, the Rf-treated mice more rapidly rotated as compared to NaCl-treated mice (p < 0.0001 for males and p = 0.011 for females). Performance of grasping reflex was better in the Rf group, only in males (p = 0.0027). In both tests, Rf-treated mice exhibited similar performance to untreated mice. No difference was found between Rf and NaCl groups for the negative righting reflex, treated pups turning less rapidly to a prone position than untreated mice.

## CONCLUSIONS

The anti-apoptotic effect of Rf on the immature mouse brain previously shown using a model of organotypic cortical slices was found in a neonatal mice lesional model. This effect is associated with a neuroprotective action and preservation of some behavioral functions in the first 12 days of life. Further experiments are required to better understand the mechanisms involved in this neuroprotective effect.

## ABS 13

### THE SafeBoosC II TRIAL: REDUCING CEREBRAL HYPOXIA WITHOUT CHANGING EARLY BIOMARKERS OF BRAIN INJURY

A.M. Plomgaard<sup>1</sup>, W. van Oeveren<sup>2</sup>, T.H. Petersen<sup>3</sup>, T. Alderliesten<sup>4</sup>, T. Austin<sup>5</sup>, F. van Bel<sup>4</sup>, M. Benders<sup>4</sup>, O. Claris<sup>6</sup>, E. Dempsey<sup>7</sup>, A. Franz<sup>8</sup>, M. Fumagalli<sup>9</sup>, C. Gluud<sup>10</sup>, C. Hagmann<sup>11</sup>, S. Hyttel-Soerensen<sup>1</sup>, P. Lemmers<sup>4</sup>, A. Pellicer<sup>12</sup>, G. Pic<sup>13</sup>

<sup>1</sup>Department of Neonatology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

<sup>2</sup>Haemoscan B.V., Groningen, the Netherlands

<sup>3</sup>Research Unit on Brain Injury Neurorehabilitation Copenhagen, Department of Neurorehabilitation, TBI Unit, Rigshospitalet, Copenhagen University Hospital, Hvidovre, Denmark

<sup>4</sup>Universitair Medisch Centrum Utrecht, Wilhelmina Children's Hospital, Utrecht, the Netherlands

<sup>5</sup>Rosie Maternity Hospital Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

<sup>6</sup>Department of Neonatology, Hopital Femme Mere Enfants, Bron, France

<sup>7</sup>Department of Paediatrics and Child Health, University College Cork, Cork, Republic of Ireland

<sup>8</sup>Department of Neonatology, University of Tübingen, Universitätsklinikum Tübingen, Tübingen, Germany

<sup>9</sup>NICU, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

<sup>10</sup>Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

<sup>11</sup>Department of Neonatology, University of Zurich, Zurich, Switzerland

<sup>12</sup>Department of Neonatology, La Paz University Hospital, Madrid, Spain

<sup>13</sup>Department of Pediatrics, Medical University of Graz, Graz, Austria

## INTRODUCTION

The SafeBoosC phase II multicentre randomised controlled trial tested the benefits and harms of monitoring cerebral oxygenation by near infrared spectroscopy (NIRS) combined with an evidence-based treatment guideline during the first 72 hours of life. The trial demonstrated a reduction of cerebral hypoxia in the experimental group compared with blindly collected NIRS-data in the control group. We here report the treatment effect on EEG (burst rate and spectral edge frequency 95%) and molecular biomarkers of brain injury in blood (S100β, brain-fatty-acid-binding-protein, and neuroketal).

## PATIENTS AND METHODS

One-hundred-and-sixty-six extremely preterm infants were randomised. EEG was recorded at 64 h of age and blood samples were collected at 6 and 64 h.

## RESULTS

One-hundred-and-thirty-three EEGs were evaluable. Burst rate did not differ between the groups (experimental 7.2 burst/min vs. control 7.7 burst/min, p = 0.51), neither did spectral edge frequency 95% (experimental 18.1 Hz vs. control 18.0 Hz). One-hundred-and-twenty-three infants had blood samples at both time-points. The change in biomarkers from 6 to 64 hours did not differ between the groups, nor did the absolute levels.

## CONCLUSIONS

Whereas cerebral hypoxia was reduced to less than half in the experimental group, neither the EEG, nor biomarkers differed between the groups.

## ABS 14

**PREDICTING WHITE MATTER DAMAGE BY EVALUATION OF THE LINEAR GROWTH RATE OF CORPUS CALLOSUM IN VERY LOW BIRTH WEIGHT INFANTS**H. Huang<sup>1</sup>, C. Chen<sup>2</sup>, H. Chou<sup>2</sup>, P. Tsao<sup>2,3</sup>, W. Hsieh<sup>2</sup><sup>1</sup>Department of Pediatrics, Taipei City Hospital, Heping FuYou Branch, Taipei, Taiwan<sup>2</sup>Department of Pediatrics, National Taiwan University Children Hospital, National Taiwan University College of Medicine, Taipei, Taiwan<sup>3</sup>Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan**INTRODUCTION**

With the improved survival of very low birth weight infants, brain injury and the subsequent neurodevelopmental disability become of enormous public health importance. Corpus callosum is a major white matter pathway and its size is proportion to axonal number and the extent of myelination. The impaired growth of corpus callosum is a recognized corollary of white matter damage in preterm infants. The aim of this study is to assess the correlation between the growth of corpus callosum in the early life and the following white matter damage in very low birth weight infants by cranial ultrasound.

**PATIENTS AND METHODS**

We prospectively recruited preterm infants with birth weight less than 1,500 grams who admitted during 2008 Sep to 2009 Dec. Those who had congenital anomalies, metabolic or infectious diseases affecting brain parenchyma were excluded. Serial cranial ultrasound were performed at 3<sup>rd</sup> day, 7<sup>th</sup> day, and 21<sup>st</sup> day of life and then every one month until the postmenstrual age (PMA) was more than 40 weeks. The thickness and length of corpus callosum were measured at each time point by standard view. Multiple logistic regression analyses were performed to evaluate the correlation between the growth of corpus callosum and the following white matter damage, which defined as periventricular leukomalacia, persistent ventriculomegaly or hydrocephalus.

**RESULTS**

Total 82 neonates were enrolled and 283 measurements were performed (37 males, 45 females; mean gestational age  $29.7 \pm 2.58$  weeks; mean birth weight  $1,193.3 \pm 298$  g). The white matter damage was identified among 14 neonates and 60 neonates had normal cranial ultrasound

results. There was no correlation between the thickness of corpus callosum and PMA, but the length of corpus callosum and vermis were strongly correlated with PMA (Pearson's  $r$  was 0.441 and 0.691, respectively). Simple linear regression revealed that the growth rate (95% confidence interval) of corpus callosum and vermis are 0.64 (0.51-0.75) mm/week and 0.75 (0.67-0.83) mm/week. However, multiple regression analysis showed that white matter damage did not alter the growth rate of corpus callosum significantly ( $p$ -value = 0.41).

**CONCLUSIONS**

The length of corpus callosum and vermis are strongly correlated with postmenstrual age before 40<sup>th</sup> gestational week in very low birth weight infants. Following white matter damage did not alter the linear growth rate of corpus callosum and vermis. Serial measurement of corpus callosum by cranial ultrasound may not predict white matter damage.

## ABS 15

**REGIONAL AND DEVELOPMENTAL SCREENING OF BILIRUBIN TOXICITY: HIPPOCAMPUS IS THE MOST SENSIBLE REGION**M. Dal Ben<sup>1</sup>, C. Tiribelli<sup>1,2</sup>, S. Gazzin<sup>1</sup><sup>1</sup>Italian Liver Foundation (Fondazione Italiana Fegato), AREA Science Park, Trieste, Italy<sup>2</sup>Department of Medical Sciences, Ospedale di Cattinara, Università degli Studi di Trieste, Trieste, Italy**INTRODUCTION**

Severe neonatal hyperbilirubinemia causes an increase of the toxic free fraction of bilirubin (Bf) and ensues in acute bilirubin encephalopathy (ABE). The severe form (kernicterus: K) causes permanent disability with motor disorders and athetosis, auditory dysfunction, memory and learning impairment. Since ABE and K present high variability in symptoms, to investigate the reason(s) of this variability we developed a new strategy allowing the identification of the most sensible brain regions and CNS developmental stages to acute bilirubin toxicity.

**PATIENTS AND METHODS**

We used the organotypic brain cultures which preserve the architecture, cellular complexity and connection of the *in vivo* nervous tissue. Hippocampus (Hip), cortex (Ctx), cerebellum



(CII), inferior (IC) and superior collicula (SC) were isolated from 2 and 8 days-old (P2 and P8) Wistar rats. After cutting (McTwain Tissue Chopper), slices were transferred on semi-porous Millicell-CM inserts and challenged for 24 hours with a free bilirubin concentration of 140 nM. Mitochondrial activity (MTT), membrane integrity (LDH release) and apoptosis (Hoechst 33258) were evaluated. Quantitative real-time PCR and immunofluorescence (GFAP) were used to assess mechanisms of damage. The neuroprotective activity of drugs was also tested.

## RESULTS

Hip, IC and Ctx presented significant higher apoptosis and, membrane leakage which was much relevant at P8 than P2 for both apoptosis (P8/P2 ratio: Hip 5.44,  $p < 0.01$ ; IC 3.35,  $p < 0.01$ ) and membrane leakage (P8/P2 ratio: Hip 7.91,  $p < 0.001$ ). SC and CII showed no toxicity. Oxidative stress (HO1 mRNA) was detected in Hip, SC and IC (3.94 fold vs. control,  $p < 0.05$ ; 3.43,  $p < 0.01$ ; 3.29  $p < 0.05$  respectively), while inflammation (TNF $\alpha$ ) only in Hip (5.53,  $p < 0.001$ ). Hip showed disruption of astrocytes network (GFAP staining), while only minor alterations were observed in other regions. Magnesium and indomethacin significantly reduced damage in Hip (both 40%,  $p < 0.001$ ), in IC, where curcumin was also effective, and Ctx.

## CONCLUSIONS

We demonstrated that bilirubin toxicity was maximal in Hip followed by IC, and surprisingly Ctx; SC was resistant to the damage. A developmental increase in sensitivity (P8 > P2) was observed in all region tested. The common mechanism seem to be excitotoxicity, followed by inflammation and oxidative stress. Differently from the *in vivo*, CII was undamaged, indicating that a longer, chronic exposure is needed to cause the effect observed *in vivo*.

## ABS 16

### EFFICACY, SAFETY AND COMORBID EVENTS DURING TRANSPORT OF ASPHYXIATED NEWBORNS TO A TERTIARY CENTRE FOR THERAPEUTIC HYPOTHERMIA

N. Carreras, M. Alsina, G. Arca, M. León, A. García-Alix

Hospital Sant Joan de Déu-Hospital Clínic, BCNatal – Centre for Maternal-Fetal and Neonatal Medicine, University of Barcelona, Barcelona, Spain

## INTRODUCTION

The management of patients with perinatal hypoxic-ischemic encephalopathy (HIE) is currently a time-dependent emergency. Most babies are born in non-tertiary neonatal units and they must be urgently transferred to a centre with a hypothermia program. In order to start therapeutic hypothermia within 6 hours of age, the current approach emphasizes both the need for early and rapid transport as well as initiating hypothermia before and during transport. We questioned if the efficacy and safety (considering comorbid events) during transport are related to the severity of the HIE.

## PATIENTS AND METHODS

A specific protocol for infant with HIE using passive hypothermia during transport was established in Catalonia in November 2009. The target temperature during transport is 33.5-35.0°C.

All newborns > 35 weeks with HIE assisted in our NICU level IIIC between January 2009 to December 2013 were prospectively collected and introduced in a specific database (DB) for HIE. This DB included transport records. The severity of the HIE was always classified immediately after admission and before starting controlled hypothermia according to a semi-quantitative scale previously reported. All patients were also monitored by aEEG, before initiating, and during controlled hypothermia.

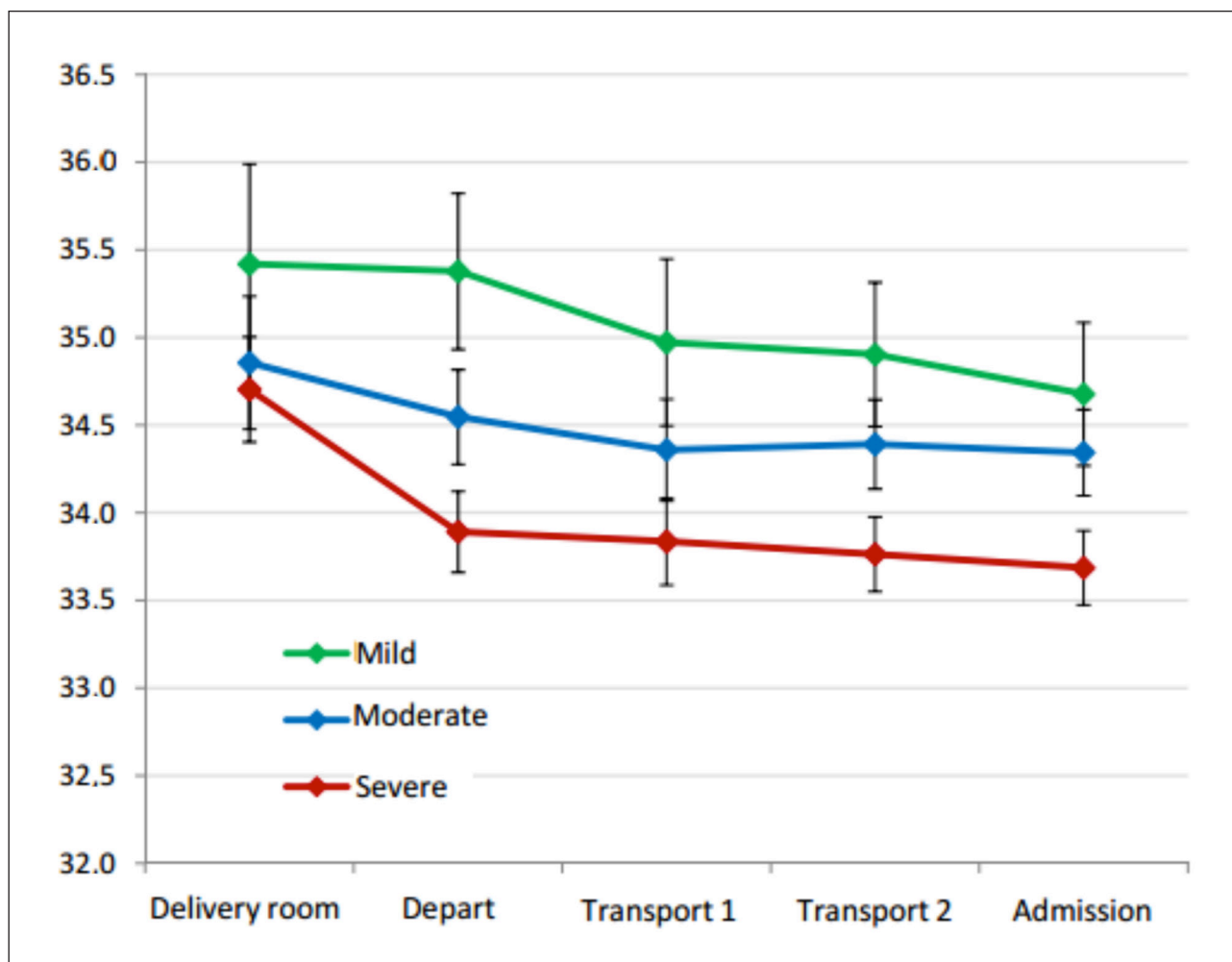
## RESULTS

Of 99 patients, 68 were outborn and transferred. There were no differences in general characteristics between inborn and outborn patients. HIE severity differed between both groups: 50% of outborn vs. 26% of inborn infants had severe HIE ( $p < 0.01$ ). Outborn group had a higher risk of death (OR 2.7). 82% were transferred without external sources of heat, one infant needed additional cooling measures and 16% an external heat source. Temperature at depart was  $34.4 \pm 1.4^\circ\text{C}$  and duration of transport was  $3.3 \pm 2$  hours. Comorbid events were present in 15% of patients; more frequent in severe HIE. The age on admission was  $5.6 \pm 2.5$  hours. On admission, 65% had temperature in the target range, 4%  $35^\circ\text{C}$ . Newborns with severe HIE had lower temperatures ( $p < 0.01$ ). Multivariate analysis showed that HIE severity and umbilical pH were factors related with temperature on admission ( $p < 0.05$ ) (Fig. 1).

## CONCLUSIONS

Our study support that passive hypothermia is effective and safe during transport, being overcooling an infrequent phenomenon. In our geographic area transferred infants have more severe HIE than those inborn. The risk of overcooling during transport is





**Figure 1 (ABS 16).** Temperature values from delivery room to admission in different forms of severity of hypoxic-ischemic encephalopathy.

higher in newborns with severe HIE, being those patients who have lower temperatures during transport.

#### ABS 17

#### BRAIN WHITE MATTER INJURY ASSOCIATED WITH NEONATAL SEIZURE AFTER ROTAVIRUS INFECTION

M.J. Kim<sup>1</sup>, S.Y. Byun<sup>2</sup>

<sup>1</sup>Department of Pediatrics, Dong-A University, Busan, Korea

<sup>2</sup>Department of Pediatrics, Pusan National University Children's Hospital, Yangsan, Korea

#### INTRODUCTION

Few cases has been reported associated with deep white matter injury after rotavirus infection. Our objective is to describe the abnormal diffusion-weighted imaging findings in 11 infants who

developed neonatal seizure during a rotavirus infection.

#### PATIENTS AND METHODS

From October 2012 through May 2014, we conducted a retrospective study of 11 newborns who were hospitalized in the NICU with neonatal seizures and rotavirus infection.

#### RESULTS

Two infants were late preterm infants (GA 35<sup>+5</sup> wks and 36<sup>+6</sup> wks) and the others were full-term infants. Apgar scores ranged between 8 and 10 at one minutes and five minutes in all infants. All infants developed clonic seizures between 4-6 days after birth. All 11 infants had normal CSF findings. No patient developed hypotension, hypoglycemia, or severe dehydration. All patients were positive for rotavirus antigen in stool. Brain diffusion-weighted imaging revealed a white matter injury in all infants. In five infants MRI was repeated at 2 weeks after onset of illness. Four infants showed cystic periventricular leukomalacia. One infant

had global neurodevelopmental delay at one year visit.

## CONCLUSIONS

We suggest an association between rotavirus infection and diffuse cerebral white matter injury characterized by neonatal seizures which developed between day 4 and 6 of postnatal life.

## ABS 18

### NOVEL PLASMA AND CEREBROSPINAL FLUID BIOMARKERS OF HYPOXIC-ISCHEMIC ENCEPHALOPATHY IDENTIFIED BY MASS SPECTROMETRY

K.J. Kyng<sup>1</sup>, A. Edhager<sup>2</sup>, C.S. Andreassen<sup>3</sup>, T.B. Henriksen<sup>1</sup>, N. Gregersen<sup>2</sup>, J. Palmfeldt<sup>2</sup>

<sup>1</sup>Perinatal Research Unit, Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark

<sup>2</sup>Research Unit for Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

<sup>3</sup>Department of Otorhinolaryngology and Head & Neck Surgery, Aarhus University Hospital, Aarhus, Denmark

## INTRODUCTION

Research in biomarkers is essential to improving the management of neonatal brain injury. Protein biomarkers may qualify the risk of neonatal brain injury, guide therapeutic decisions and improve prognostication in hypoxic-ischemic encephalopathy (HIE). Current candidate biomarkers originate from adult studies of brain injury and we hypothesize that proteins specific to neonatal brain injury may better characterize HIE. The aim of this study was to identify novel protein biomarkers of HIE using mass spectrometry in a piglet model. We sampled both cerebrospinal fluid (CSF) and plasma to examine the relationship between biomarkers found in these two compartments.

## PATIENTS AND METHODS

Newborn piglets (n = 6, 18 hours old) were instrumented for continuous monitoring. HIE was induced by lowering FiO<sub>2</sub> to 4% over a 45-minute period to suppress aEEG to < 7  $\mu$ V. Plasma and CSF were collected before hypoxia and two hours after. Proteins were analyzed by nanoLC-MS/MS followed by bioinformatics analyses. Significantly altered proteins were to meet three criteria; 1) a minimum of two unique quantified peptides, 2) p-value < 0.05, and 3) fold change alteration of  $\geq$  two times the global standard error of mean (22% and 41% for plasma and CSF, respectively).

Validation of detected proteins in a new round of experiments by selected reaction monitoring mass spectrometry (SRM-MS) is ongoing.

## RESULTS

Approximately 100 proteins in plasma and 70 proteins in CSF were significantly altered after hypoxia-ischemia. The proteins were involved in essential cellular pathways, such as the antioxidant system, cell proliferation, cell structure, and apoptosis. The most up-regulated protein in CSF was S100A, known to be highly regulated during tissue injury. Proteins co-upregulated in CSF and plasma included IGJ and SERPINA3, whereas keratins KRT1 and 10 were downregulated in CSF but upregulated in plasma. In plasma, FABP1 was the highest upregulated protein, and other upregulated proteins included HSPB1, HSPA6 and SOD1.

## CONCLUSIONS

Untargeted LC-MS enabled characterization of proteome changes following hypoxia-ischemia in a standardized newborn piglet model. A set of new candidate biomarkers for HIE, as well as proteins from pathways already known to be involved in the response to HIE were identified. Candidate biomarkers will be validated in additional experimental animals and tested in clinical samples from newborn children suffering from HIE.

## ABS 19

### RISK OF HYPOCAPNIA IN ASPHYXIATED NEWBORNS TREATED WITH EARLY THERAPEUTIC HYPOTHERMIA

E. Szakmar<sup>1</sup>, A. Jermendy<sup>1</sup>, A. Nagy<sup>2</sup>, A. Szell<sup>2</sup>, A. Berenyi<sup>2</sup>, B. Bundzsity<sup>2</sup>, A. Cseko<sup>1</sup>, G. Bokodi<sup>1</sup>, D. Kelen<sup>1</sup>, U. Meder<sup>1</sup>, Zs. Somogyvari<sup>2</sup>, M. Szabo<sup>1</sup>

<sup>1</sup>1<sup>st</sup> Department of Paediatrics, Semmelweis University, Budapest, Hungary

<sup>2</sup>Neonatal Emergency & Transport Services of Peter Cerny Foundation, Budapest, Hungary

## INTRODUCTION

Therapeutic hypothermia (HT) has been shown to improve neurodevelopmental outcome in term newborns with moderate to severe hypoxic ischaemic encephalopathy (HIE). An observational study demonstrated that early cooling offers more neuroprotective effect, and other studies suggest that early cooling is feasible and safe even during neonatal transport. However cooling causes a reduction in metabolic rate and

may increase the risk of hypocapnia, which is toxic for the injured brain.

#### PATIENTS AND METHODS

Our aim was to evaluate the association between early cooling and hypocapnia. In this retrospective study, term newborns with moderate to severe HIE were included. HIE was defined according to the TOBY criteria. Neonates who received intensive care plus active cooling during transport ( $n = 87$ , early cooled group) were compared to neonates who received intensive care alone during transport ( $n = 39$ , control group). Prevalence of hypocapnia was analysed in both groups. Hypocapnia was defined as temperature corrected  $p\text{CO}_2$  value less than 35 mmHg in blood gas samples. Data were compared by non-parametric statistical tests and logistic regression modelling.

#### RESULTS

Baseline patient characteristics and clinical data were similar in the two groups. The target temperature of hypothermia treatment ( $33\text{--}34^\circ\text{C}$ ) was reached at a median of 1.83 [1.3-2.6] hours of life in the early cooled group; and at 5.0 [3.2-6.8] hours in the control group ( $p < 0.001$ ). More neonates developed hypocapnia in the early cooled group compared to the control group (39.08% vs. 20.51%,  $p = 0.04$ ). Multiple logistic regression analysis adjusting for blood gas values and parameters of mechanical ventilation showed that hypothermia is a strong predictor of hypocapnia during transport (OR 3.24; 95%CI 1.1-11.0). In addition, sedation and/or muscle relaxation had a tendency to protect against hypocapnia in neonates with HIE (OR 0.37; 95%CI 0.1-1.0), most likely because of their negative effects on spontaneous respiratory efforts.

#### CONCLUSIONS

Early induction of therapeutic hypothermia may increase the risk of hypocapnia in asphyxiated infants during neonatal transport. Therefore, a tighter control of ventilation, and possibly sedation/muscle relaxation should be considered in selected cases to prevent hypocapnia. Further clinical trials are warranted to determine if early HT could elicit more favourable neurodevelopmental outcome with rigorous  $p\text{CO}_2$  control.

#### ABS 20

#### INCIDENCE AND RISK FACTORS OF INTRAVENTRICULAR HEMORRHAGE IN INBORN AND OUTBORN INFANTS IN THIRD LEVEL HOSPITALS BEFORE 32 WEEKS OF PREGNANCY

D. Szpecht, M. Szymankiewicz, J. Gadzinowski

*Department of Neonatology, Medical University of Poznan, Poznan, Poland*

#### INTRODUCTION

Intraventricular hemorrhage (IVH) affects 15-20% of babies born before 32 weeks of pregnancy. Making appropriate recommendations for dealing with infant born less than 32 weeks of gestation aimed at reducing the incidence of IVH is still needed. The study aim was to determine the incidence and analyze risk factors of IVH stage 3 and 4 in infants inborn and outborn third level hospitals before 32 weeks of pregnancy.

#### PATIENTS AND METHODS

The retrospective analysis of 267 preterm babies (24 to 32 weeks of gestation) hospitalized in 2011-2013 at Department of Neonatology, Poznan University of Medical Sciences, was performed. Of the 267 infants, 223 (83.5%) were inborn and 44 (16.5%) were outborn. The diagnosis of IVH was confirmed by ultrasound scans according to Papile's criteria. Stage 3 and 4 of IVH was confirmed in 14 of 38 (36.8%) newborns from 23-24 weeks of gestation; 21 of 86 (24.4%) from 25-26 weeks of gestation; 11 of 64 (17.2%) from 27-28 weeks of gestation; 9 of 54 (16.6%) from 29-30 weeks of gestation and 1 of 25 (4%) from 31-32 weeks of gestation.

#### RESULTS

Stage 3 and 4 of IVH confirmed in 42 (18.8%) inborn and 14 (31.8%) outborn infants ( $p = 0.05$ ). Incidence of IVH stage 3 and 4 was higher in children: with less use of prenatal steroids (62.5% vs. 37.5%  $p = 0.014$ ), treated with crystalloids (bolus 10-15 ml/kg) and/or catecholamines for hypotension (83.9% vs. 16.1%,  $p = 0.002$ ) and treated with  $\text{NaHCO}_3$  due to acidosis (80.4% vs. 19.6%,  $p = 0.003$ ). Using multivariable analysis confirmed 10-fold higher risk of IVH stage 3 and 4 in infants outborn, before 32 weeks of pregnancy without prenatal steroids, vaginally delivered, treated with crystalloids (bolus 10-15 ml/kg) and/or catecholamines for hypotension, with symptoms of blood clotting disorders and/or thrombocytopenia compare to newborns without these risk factors.

#### CONCLUSIONS

The risk of IVH stage 3 and 4 was the greater the lower the gestational age. The use of appropriate prophylaxis of perinatal steroids in all pregnant women at risk of preterm birth, promotion of birth before 32 weeks of pregnancy via caesarean section, minimalization of outborn births, limiting the indications for the use of catecholamines and

crystalloids for hypotension treatment significantly reduce the incidence of IVH stage 3 and 4.

## ABS 21

### IMPAIRMENT OF OLIGODENDROGLIAL DEVELOPMENT *IN VITRO* AT 21% O<sub>2</sub> IS ATTENUATED BY STABILIZATION OF HYPOXIA-INDUCIBLE FACTOR 1 $\alpha$

C. Brill, R. Herrmann, C. Bühner, T. Schmitz

*Department of Neonatology, Charité University Medicine Berlin, Berlin, Germany*

#### INTRODUCTION

The development of immature oligodendroglia is highly sensitive to oxidative stress and increased O<sub>2</sub> levels. We have previously shown that the 21% O<sub>2</sub> sustained in standard cell cultures are worsening the development of oligodendroglial lineage cells as compared to lower levels of 5% O<sub>2</sub>. However, the mechanisms through which higher O<sub>2</sub> concentrations interfere with oligodendroglial development remain unclear. Since hypoxia-inducible factor (HIF) has been described to be involved in oligodendroglial differentiation, we hypothesized that dysregulation of HIF is contributing to O<sub>2</sub>-induced developmental impairment in oligodendroglia.

#### PATIENTS AND METHODS

In cell lysates of purified rat oligodendroglial precursor cell (OPC) cultures, we determined levels of oxidative stress induced by 48 hrs incubation at 21% and at 5% O<sub>2</sub> by nitrotyrosine Western blot of cell lysates. Oligodendroglial development was analyzed by immunocytochemistry (ICC) using antibodies for A2B5 and O4 to label OPCs and immature oligodendroglia, respectively, and Ki67 and PCNA to label proliferation. Sholl analysis with ImageJ software was used in O4<sup>+</sup> immature oligodendroglia to evaluate arborisation and process complexity. The role of HIF-dependent dysregulation of oligodendroglial development was analyzed by supplementation of cobalt chloride which is known to increase HIF-1 $\alpha$  levels by inhibition of prolyl hydroxylases.

#### RESULTS

Culture conditions with 5% O<sub>2</sub> improved proliferation, oligodendroglial cell numbers and specific gene expression in comparison to 21% O<sub>2</sub>. Nitrotyrosine levels were increased in OPC cultures obtained at 21% O<sub>2</sub>. Notably, in cultures at 21% O<sub>2</sub>, stabilization of HIF-1 $\alpha$  by cobalt chloride

significantly improved the development of O4<sup>+</sup> immature oligodendroglia signified by process complexity and cell surface analysis which were then similar to cultures kept at 5% O<sub>2</sub>.

#### CONCLUSIONS

Degradation of HIF-1 $\alpha$  is significantly involved in maldevelopment of oligodendroglia caused by 21% O<sub>2</sub>.

## ABS 22

### BODY CORE TEMPERATURE: A NOVEL BIOMARKER FOR HYPOXIC-ISCHAEMIC ENCEPHALOPATHY

D. Jayasinghe<sup>1</sup>, L. Wilcox<sup>2</sup>

<sup>1</sup>NICU, Nottingham City Campus, Nottingham, UK

<sup>2</sup>University of Nottingham School of Medicine, Queens Medical Centre, Nottingham, UK

#### INTRODUCTION

The aim of this study was to investigate whether the core temperature profiles of babies born after hypoxic-ischaemic (HI) delivery were different between those who were neurologically normal and those who developed encephalopathy (HIE). Therapeutic hypothermia (TH) is an effective treatment for neonatal HIE. Historical data suggested impaired thermal adaptation following perinatal asphyxia, using classical definitions of hypoxic ischaemia. It is our institution's standard practice to admit, observe and passively cool babies who have evidence of HI delivery and neurological dysfunction but without HIE. If during observation HIE develops then TH is started, otherwise the baby is rewarmed.

#### PATIENTS AND METHODS

A cohort retrospective nursing chart analysis of infants who underwent passive-cooling during 2012-13 was conducted. We identified infants from the clinical database who were  $\geq 36$  week's gestation,  $< 6$  hours age and admitted for observation after HI delivery. All infants met at least one of the following inclusion criteria: 10 minute Apgar score  $\leq 5$ , resuscitation at 10 minutes, acidosis (pH  $< 7.00$ ) or base deficit ( $\geq 16$  mmol/L) within 60 minutes. Subjects were divided into two groups, the HIE group had a clinical diagnosis of HIE after observation, the controls met the criteria but were not diagnosed with HIE (control group). Hourly temperature measurements were abstracted from nursing charts. The median temperature for each

group was calculated at admission and each hour after admission.

## RESULTS

A total of 40 babies were identified (HIE = 20, control = 20). There was a trend of lower temperature profiles for neonates with HIE compared to controls for the first 3 hours. The median temperatures at admission to the neonatal intensive care unit and at two hours of age were statistically significantly lower ( $p = 0.023$ ,  $p = 0.008$ ) compared to controls (**Tab. 1, Fig. 1**).

**Table 1 (ABS 22).** Main characteristics and temperature values in newborns who developed hypoxic-ischaemic encephalopathy (HIE) and controls.

	HIE	Control
<b>Gestational age</b>	39 w 4 d	40 w 1 d
<b>Birth weight (g)</b>	3,195	3,540
<b>Admission age (mins)</b>	20	21
<b>Temperature (°C) (median, IQR)</b>		
<b>Admission</b>	36.0 (35.3, 36.5)	36.6 (36.4, 36.9) <sup>a</sup>
<b>1 hour age</b>	35.9 (34.7, 36.5)	36.8 (35.8, 37.1)
<b>2 hours age</b>	36.1 (36.0, 36.3)	37.1 (36.7, 37.2) <sup>a</sup>
<b>3 hours age</b>	35.6 (34.2, 36.3)	35.6 (34.7, 36.4)

<sup>a</sup> $p < 0.05$ .

## CONCLUSIONS

Core body temperature was different between infants with and without HIE after HI delivery. There were no other significant differences. Body temperature measurements have shown potential for use as a clinically useful biomarker of HIE. Further large, prospective trials are needed to test sensitivity and specificity before its clinical effectiveness can be determined.

## ABS 23

### APGAR SCORES PREDICT ENCEPHALOPATHY MORE THAN BIOCHEMICAL MARKERS

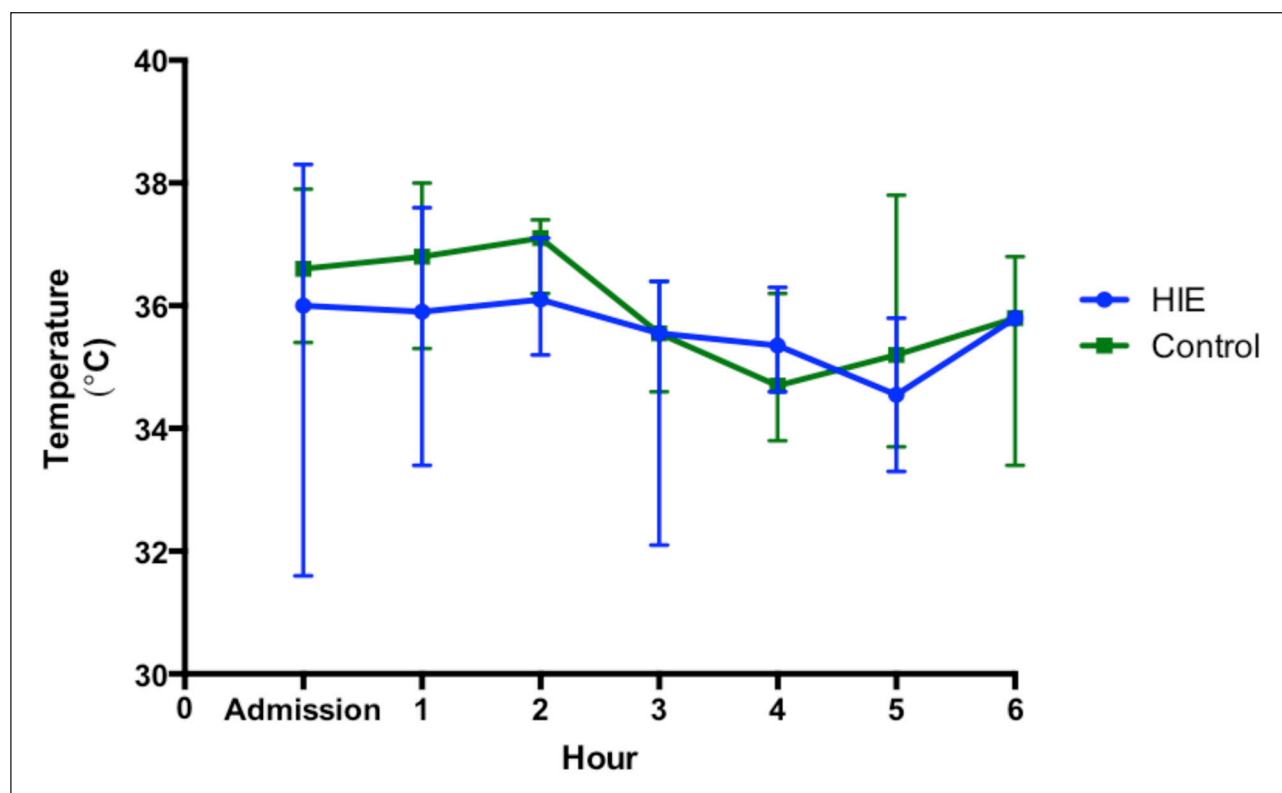
R. Jones<sup>1</sup>, A. Heep<sup>1,2</sup>, D. Odd<sup>1,2</sup>

<sup>1</sup>Neonatal Intensive Care Unit, North Bristol NHS Trust, Southmead Hospital, Bristol, UK

<sup>2</sup>University of Bristol, School of Clinical Sciences, Bristol, UK

## INTRODUCTION

Therapeutic hypothermia (TH) for infants with hypoxic-ischaemic encephalopathy (HIE) is a time-critical emergency and should be started within 6 hours after the insult. Evidence for how to best



**Figure 1 (ABS 22).** Body core temperature in the first hours of life in newborns who developed hypoxic-ischaemic encephalopathy (HIE) and controls.



identify infants who might benefit from TH is lacking. In accordance with a regional guideline, all infants with a cord pH of  $< 7$  are admitted for 6 hours of cerebral function monitoring (CFM) in order to correctly identify infants with HIE and offer timely TH.

The aim of our study was to identify the best perinatal markers to predict which infants would develop HIE after perinatal asphyxia. Furthermore, we also wanted to examine the morbidities seen in infants that did not develop HIE.

#### PATIENTS AND METHODS

This is a retrospective observational cohort study which was carried out at the Neonatal Intensive Care Unit at Southmead Hospital, Bristol, UK. All inborn patients born between 01/01/2012 and 31/03/2014 at  $> 36$  completed weeks gestation with a pH of  $< 7$  on cord or baby's blood within 1 hour of birth were eligible for inclusion. Infants were identified and data regarding their hospital stay was obtained from their electronic patient record.

The primary outcome measure for the study was the predictive values of clinical and biochemical measures to identify which infants would progress to develop HIE. ROC curves were developed for the perinatal measures and the area under the curve (AUC) used as the measure of prediction.

#### RESULTS

We identified a total of 79 eligible babies. Infants with abnormal early CFM, qualifying for TH were considered to have HIE, whereas babies with normal early CFM recording were labelled as non-HIE. Infants were analysed according to these subgroups (**Tab. 1**). 13 (16.5%) were diagnosed with HIE and received TH while 66 babies (83.5%) were non-HIE. There was evidence that cord pH, base excess, 1, 5 and 10 minute Apgar score and need for IPPV at 10 minutes were associated with development of HIE (all p-values  $< 0.05$ ). Lactate, Troponin T and ALT, but not CK, LDH AST or GGT were associated with development of HIE (using  $p < 0.05$  as a cut off). The highest AUC measure was associated with the Apgar score at 5 minutes (0.89), while pH (0.75) and Troponin level (0.81) also showed high values. 62.1.2% of non-HIE babies were treated for probable sepsis and 28.8% were hypoglycaemic on admission.

#### CONCLUSIONS

We found in our study population that early measures of birth condition such as Apgar scores, level of acidosis, need for IPPV at 10 minutes and early Troponin levels are predictive of the development of HIE. A high proportion of non-HIE infants were treated for probable sepsis and/or initial hypoglycaemia which suggests

**Table 1 (ABS 23).** Birth conditions and biochemical measures in the two subgroups.

	n	Non-HIE (n = 66)	HIE (n = 13)	Area under ROC	p-value
<b>Birth condition</b>					
Worst cord pH (median, IQR)	77	6.96 (6.89-6.98)	6.90 (6.80-6.91)	0.7521	0.007
Worst cord BE (median, IQR)	72	13.35 (12.3-15.6)	16.1 (13.1-17.8)	0.6573	0.112
Apgar score 1 min (median, IQR)	79	3 (4-7)	1 (1-4)	0.8485	$< 0.001$
Apgar score 5 min (median, IQR)	77	9 (7-9)	5 (3-7)	0.8864	$< 0.001$
Apgar score 10 min (median, IQR)	79	9 (8-10)	4 (0-7)	0.8217	$< 0.001$
IPPV at 10 mins (n, %)	79	46 (69.7%)	13 (100%)	0.6515	0.022
<b>Biochemical measures</b>					
Lactate (mmol/L) (median, IQR)	35	8.8 (5.4-12.9)	16.3 (8.8-18.8)	0.7567	0.070
Troponin T (ng/L) (median, IQR)	31	93 (80-114)	145 (114-649)	0.8114	0.004
CK (IU/L) (median, IQR)	23	1,004 (770-1,257)	1,163 (948-2,737)	0.6742	0.157
LDH (IU/L) (median, IQR)	29	16 (10-22)	19 (10-65)	0.6364	0.225
ALT (IU/L) (median, IQR)	44	19 (12-33)	65 (20-176)	0.7767	0.004
AST (IU/L) (median, IQR)	12	88 (77-112)	219 (60-245)	0.6857	0.291
GGT (IU/L) (median, IQR)	7	110 (91-398)	86 (45-186)	0.6667	0.480
CRP (IU/L) (median, IQR)	65	1.8 (0.8-4.2)	2 (2-2)	0.5220	0.846
Alk Phos (IU/L) (median, IQR)	44	147 (131-206)	187 (142-243)	0.6576	0.102

that careful evaluation of infants with perinatal acidosis might be warranted.

## ABS 24

### REFERENCE VALUES FOR THE NEUROPEPTIDE SECRETONEURIN IN HEALTHY TERM NEWBORNS

A. Schmid<sup>1</sup>, M. Höck<sup>1</sup>, A. Posod<sup>1</sup>, M. Urbanek<sup>1</sup>, R. Fischer-Colbrie<sup>2</sup>, V. Neubauer<sup>1</sup>, U. Kiechl-Kohlendorfer<sup>1</sup>, E. Griesmaier<sup>1</sup>

<sup>1</sup>Department of Paediatrics II, Neonatology, Innsbruck Medical University, Innsbruck, Austria

<sup>2</sup>Department of Pharmacology, Innsbruck Medical University, Innsbruck, Austria

## INTRODUCTION

Secretoneurin (SN) is a polypeptide, which is produced by endocrine, neuroendocrine and neuronal cells. It has several biological effects including modulation of inflammatory responses and neurotransmission. Interestingly, SN levels are elevated in adult animal models of brain ischaemia and in human adults suffering from brain injury after cardiopulmonary resuscitation. SN could be used as a biomarker to predict neurological outcome and might be also of interest in newborn infants with brain injury. At present there are no data available about SN levels in newborns. The aim of this study was to determine reference values for SN in healthy term-newborns.

## PATIENTS AND METHODS

During the study period, starting in November 2013 until April 2014 a total number of 131 healthy term-newborns (65 males) were prospectively enrolled in this study. SN levels were assessed by radioimmunoassay in cord blood and at 48 hours after birth. Maternal and neonatal data included mode of delivery, duration of rupture of membranes, pathological cardiotocogram, duration of fetal expulsion (hours), duration of labour (hours), umbilical cord pH, Apgar score, sex, gestational age (full weeks of gestation) and birth weight (grams).

## RESULTS

In umbilical cord blood SN serum level was  $147.97 \pm 84.30$  fmol/ml and decreased to  $96.68 \pm 41.55$  fmol/ml at 48 hours after birth. Birth mode significantly influenced SN levels in umbilical cord blood, meaning that SN levels were highest in infants born per vacuum extraction ( $218.59$

$\pm 97.96$  fmol/ml) compared to infants born spontaneously ( $163.53 \pm 58.31$  fmol/ml;  $p = 0.001$ ) and infants born per c-section ( $85.39 \pm 66.42$  fmol/ml;  $p = 0.010$ ). Spontaneously born infants also showed significantly higher SN levels in umbilical cord blood compared to infants born per c-section ( $p = 0.001$ ). Infants with a pathological cardiotocogram (CTG) showed significantly higher SN serum levels in umbilical cord blood compared to infants with a normal CTG ( $193.65 \pm 83.22$  fmol/ml vs.  $116.67 \pm 70.17$  fmol/ml;  $p < 0.001$ ). We found no influence on the other above mentioned maternal and neonatal variables on SN serum levels.

## CONCLUSIONS

We are the first to provide data on SN levels in healthy term-newborns. Neonatal factors including birth mode and cardiotocogram that could serve as a marker of neonatal stress had an effect on SN levels. This data provide a basis for further studies evaluating the role of SN as a potential biomarker in neonatal brain injury.

## ABS 25

### EFFECTS OF VENTILATION ON THE CEREBRAL CORTEX FOLLOWING INTRA-UTERINE INFLAMMATION IN PRETERM LAMBS

A. Atik<sup>1</sup>, B. Skiöld<sup>2</sup>, S. Barton<sup>1</sup>, J. Pearson<sup>3</sup>, Q. Wu<sup>3</sup>, V. Zahra<sup>1</sup>, A. Moxham<sup>1</sup>, S. Hooper<sup>1</sup>, M. Tolcos<sup>1</sup>, R. Galinsky<sup>4</sup>, G. Polglase<sup>1</sup>

<sup>1</sup>MIMR-PHI Institute of Medical Research, Melbourne, VIC, Australia

<sup>2</sup>Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden

<sup>3</sup>Department of Physiology & Monash Biomedical Imaging, Monash University, Clayton, Melbourne, VIC, Australia

<sup>4</sup>Department of Physiology, University of Auckland, Auckland, New Zealand

## INTRODUCTION

Ventilation of preterm lambs causes cerebral white matter inflammation and injury, which is exacerbated by intrauterine inflammation. However, the effects on the cerebral cortex are not known. Our aim was to examine the effects of a protective or injurious ventilation strategy on the cerebral cortex with and without prior exposure to intrauterine inflammation.

## PATIENTS AND METHODS

Newborn lambs (0.85 gestation) were ventilated using either an injurious ventilation ( $n =$

6) strategy for 15 min followed by a protective ventilation strategy for 75 min or a protective ventilation (n = 5) strategy for 90 min. Additional two groups were exposed to intra-amniotic lipopolysaccharide (LPS; 10 mg; to induce intrauterine inflammation) 7 days prior to delivery and injurious (n = 5) or protective (n = 6) ventilation. At autopsy, sections of the cerebrum were immunohistochemically stained to identify microglia (Iba-1), astrocytes (GFAP), neurons (NeuN) and vascular protein extravasation (sheep serum) within the cerebral cortex. Results were compared using 2-way ANOVA.

## RESULTS

There were no significant differences between groups in the overall density of microglia or astrocytes. LPS exposure reduced the overall density of neurons compared to controls (PLPS = 0.02) with ventilation strategy having little effect. 50% of LPS exposed lambs had vascular protein extravasation; no vascular protein extravasation was observed in the ventilation only groups.

## CONCLUSIONS

Intrauterine inflammation prior to ventilation reduced neuronal cell density and increased vascular protein extravasation. A protective ventilation strategy did not reduce brain injury in LPS exposed lambs.

## ABS 26

### CEREBRAL ULTRASOUND FINDINGS DURING THERAPEUTIC HYPOTHERMIA FOR NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY: VALIDATION OF A NEW CLASSIFICATION SYSTEM

A. Graca<sup>1</sup>, J. Barreira<sup>2</sup>, C. Santos<sup>3</sup>, C. Moniz<sup>1</sup>

<sup>1</sup>Neonatal Intensive Care Unit, Department of Pediatrics, Hospital de Santa Maria, CHLN, Lisbon Academic Medical Centre, Lisbon, Portugal

<sup>2</sup>Department of Neuroradiology, Hospital Egas Moniz, CHLO, Lisbon, Portugal

<sup>3</sup>Department of Neuroimaging, Hospital Santa Maria, CHLN, Lisbon Academic Medical Centre, Lisbon, Portugal

## INTRODUCTION

MRI has a central role on defining outcome of neonatal hypoxic-ischemic encephalopathy. Nevertheless, early outcome prediction during hypothermia is important, mainly in severe cases. Clinical status, aEEG and NIRS have important roles in that matter, but cerebral ultrasound (cUS),

despite being performed regularly, is believed to have a weak prognostic value. We aim to evaluate the ability of our unpublished cUS classification system to predict significant MRI changes on the second week of life.

## PATIENTS AND METHODS

We reviewed cUS and MRI images from all patients treated with hypothermia at our NICU. cUS were classified according to a local classification system: A) Basal ganglia-thalami (BGT) echogenicity; B) Visualization of the internal capsule (IC); C) Periventricular white matter (PWM) echogenicity; D) Cortico-subcortical differentiation. MRI findings were classified according to Rutherford classification (Lancet Neurol, 2010). Patients were then reclassified as having lesions that predict a favorable outcome (normal/subtle changes) or an unfavorable outcome (moderate/severe changes) both for cUS and MRI. McNemar test was performed to compare paired cUS/MRI outcome classifications. Sensibility, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

## RESULTS

44 patients were studied. Global cUS classification was unable to predict accurately MRI changes ( $p < 0.02$  on McNemar test). After excluding changes that are more difficult to classify on cUS, as periventricular white matter and cortico-subcortical differentiation, we found that our cUS classification was able to predict MRI changes ( $p = 0.629$  on McNemar test), showing good specificity (77%) and negative predictive value (70%), but lower sensibility (29%) and positive predictive value (36%) for predicting adverse findings on MRI.

## CONCLUSIONS

During hypothermia it is important to use a multimodal approach for outcome prediction. In this setting, we found that absence of significant changes on central gray matter using our proposed cUS classification system was found to be a good additional tool to predict favourable findings on MRI, thus complementing data provided by aEEG and NIRS. Nevertheless, MRI after completing treatment remains essential to predict outcome.

## ABS 27

### A NEW TRANSLATIONAL MODEL OF PERINATAL ASPHYXIA REVEALS LASTING BEHAVIORAL DEFICITS

A. Kerenyi<sup>1,2</sup>, E. Sipos<sup>1</sup>, P. Bakos<sup>1</sup>, K. Demeter<sup>1</sup>, P. Pottyondi<sup>1</sup>, J. Haller<sup>1</sup>, M. Szabo<sup>3</sup>, K. Kaila<sup>2</sup>, E. Mikics<sup>1</sup>, A. Denes<sup>1</sup>, A. Fekete<sup>1</sup>

<sup>1</sup>*Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary*

<sup>2</sup>*University of Helsinki, Department of Biosciences and Neuroscience Center, Helsinki, Finland*

<sup>3</sup>*1<sup>st</sup> Department of Pediatrics, Semmelweis University, Budapest, Hungary*

## INTRODUCTION

Translating preclinical findings into clinical benefit represents a largely unresolved problem in perinatal asphyxia research. While therapeutic hypothermia now offers the first efficient therapy for birth asphyxia, there are hundreds of interventions which failed in the clinic after showing benefits in preclinical models. One of the reasons for this major gap in translation is the lack of adequate animal models. Our goal was to test a novel translational rodent model of perinatal asphyxia using clinically relevant biomarkers.

## PATIENTS AND METHODS

We adopted the Kaila model of birth asphyxia (Helmy et al., *Annals of Neurology*, 2011), where an asphyxic gas mixture (4% O<sub>2</sub> & 20% CO<sub>2</sub>) is used to mimic the clinical condition without any surgical intervention. 128 Wistar rat pups from both sexes on postnatal day 6 or 7 were exposed to the asphyxic gas mixture or room air for 15 mins in a temperature-controlled chamber (37-37.5°C) and then returned to their dams. No measures of resuscitation were taken. Serum and tissue samples were collected at 3, 4, 8, and 24 hours after asphyxia for analysis of laboratory parameters, cytokines, mRNA markers of kidney injury and brain histology. A group of animals were allowed into adulthood and were tested for sensory-motor, cognitive and behavioral deficits.

## RESULTS

Overall mortality was approximately 25% during asphyxia, while no mortality was observed afterwards. Immunohistochemistry showed increased microglial activation in the hippocampus 24 h after asphyxia. Neuronal apoptosis and white matter injury were not observed and the animals did not show significant sensory-motor deficits. However, long term functional testing identified the development of anxiety- and impulsivity-like behavior in the Elevated Plus Maze and Operant Learning paradigms, without significant deficits in learning. We have also investigated markers of multi-organ injury. Laboratory parameters (Se Na,

carbamide, creatinine, GOT, GPT) did not show significant changes after asphyxia. Changes were not detected in serum and tissue cytokine levels. In contrast, mRNA transcripts of kidney injury markers NGAL and KIM-1 showed a significant increase 4 h and 24 h after asphyxia, respectively.

## CONCLUSIONS

In a new, translational model we showed that neuroinflammation develops after asphyxia in the absence of acute neuronal injury and it is associated with behavioral alterations in adulthood. Upregulation of kidney injury markers indicates systemic changes, although no evidence for multi-organ injury was found. This new model could provide clinically relevant insight into the systemic changes and diverse behavioral symptoms following birth asphyxia.

## ABS 28

### EFFECT OF EARLY NUTRITION ON PRETERM CEREBRAL MATURATION AND BRAIN INJURY REFLECTED BY MR-IMAGING AT TERM

L. Beauport<sup>1,2</sup>, J. Schneider<sup>1</sup>, P. Hagmann<sup>3</sup>, M. Faouzi<sup>4</sup>, C.J. Fischer Fumeaux<sup>1</sup>, A.C. Truttmann<sup>1</sup>

<sup>1</sup>*Clinic of Neonatology and Follow-up, Department of Pediatrics, University Hospital Center and University of Lausanne, Lausanne, Switzerland*

<sup>2</sup>*Division of Neonatology, Department of Pediatrics, Centre Hospitalier Chrétien, Site St-Vincent, Rocourt, Belgium*

<sup>3</sup>*Department of Radiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland*

<sup>4</sup>*Biostatistics, Institute of Social and Preventive Medicine, Lausanne, Switzerland*

## INTRODUCTION

Although early optimal nutrition has shown positive effects on neurodevelopment of preterm infants, its effects on cerebral maturation and injury remain poorly understood. Our objective was to study the association between nutritional intakes during the first two weeks of life and the cerebral MRI performed at term equivalent age in very preterm infants.

## PATIENTS AND METHODS

Preterm infants < 30 weeks were prospectively recruited. Clinical and nutritional intakes were collected. Cumulative doses of proteins, lipids, carbohydrates and energy delivered from day 1 to 14 were calculated. A MRI (3T) at term equivalent age was performed using T1 and T2 sequences. Scores according to Kidokoro1 were assessed for



severity of the white and grey matter lesions and maturational level, resulting in a global score. Patients were then divided in group 1 with normal or mild global score (score < 75<sup>th</sup> percentile) and group 2 with moderate and severe global score group (score ≥ 75<sup>th</sup> percentile). The association between nutritional intakes and grade of the MR score was analyzed by uni- and multivariate logistic regression.

## RESULTS

We included 42 patients with a mean (SD) of 27.5 (1.3) gestational weeks and birth weight of 920 (215) g. The male:female ratio was 0.82. The median MRI global score was 4 and 75<sup>th</sup> percentile was 6, separating group 1 (score 0-5, n = 27) from group 2 (score 6-12, n = 15). In univariate analysis, high nutrient intakes were associated with a significantly lower risk of having a MR score ≥ 6 (OR [95%CI] energy: 0.99 [0.98-0.99], lipids: 0.89 [0.83-0.97], carbohydrates: 0.95 [0.91-0.99]). Similar non significant trend was observed for proteins. Early onset sepsis increased the risk of having a MR score ≥ 6 (OR 10.9 [1.88-63.7]). When adjusted for sepsis, the effect of nutritional intakes was stronger (OR [95%CI] energy: 0.16 [0.04-0.57], lipids: 0.27 [0.10-0.71], carbohydrates: 0.46 [0.24-0.48]). Accordingly, an increase of 10 kcal/day would decrease the risk of having a high MR score by 71%.

## CONCLUSIONS

This study reveals a crucial role of early nutrition on cerebral development evaluated by MRI at term in premature infants. According to these results, optimizing nutritional intakes improves brain development and could even reduce negative impact of early aggression as sepsis. The impact on long term neurodevelopmental outcome has to be confirmed in further studies and in more vulnerable and cerebrally injured populations.

## ABS 29

### NEURITE OUTGROWTH IN RESPONSE TO CEREBROSPINAL FLUID DERIVED FROM NEC-SENSITIVE PRETERM PIGS

J. Sun<sup>1</sup>, S. Pankratova<sup>2</sup>, D.E.W. Chatterton<sup>1,3</sup>, P.T. Sangild<sup>1</sup>

<sup>1</sup>Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark

<sup>2</sup>Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark

<sup>3</sup>Department of Food Science, University of Copenhagen, Copenhagen, Denmark

## INTRODUCTION

Preterm infants suffer from an immature intestine and a delayed brain development. Early enteral feeding is important to stimulate gut maturation, but aggressive feeding, especially with formula, may induce inflammation and necrotizing enterocolitis (NEC). It is not known whether this would affect the developing brain. In this pilot study, we used primary rodent hippocampal neurons, which are essential for cognition and learning, as an *in vitro* model to study neuronal differentiation following administration of cerebrospinal fluid (CSF) from preterm pigs fed either formula or pasteurized human donor milk.

## PATIENTS AND METHODS

Samples of CSF were collected from preterm pigs fed preterm formula (n = 5) or human donor milk (n = 8) via cisternal puncture immediately after euthanasia on days 5-8 after birth. Regions of the gut (stomach, intestine and colon) were graded for NEC lesions, and pigs with a score of ≥ 4 were diagnosed as having NEC. Hippocampal neurons isolated from Wistar rats at embryonic day 19 were cultured and stimulated with serially diluted CSF samples for 24 h. Neurons were then fixed and stained with the neuronal growth cones marker, GAP-43. The length of neurites per cell was estimated by analysis of fluorescent images employing a stereological approach.

## RESULTS

The pasteurized human donor milk group had a significantly lower NEC score specifically in the colon compared to the formula group ( $1.13 \pm 0.35$  vs.  $5.00 \pm 0.71$ ,  $p < 0.001$ ), indicating that colon NEC incidence is feeding-dependent. Further evaluation of neurite outgrowth, used as an indicator of neuronal differentiation, showed that 7 of 8 CSF samples (87.5%) from NEC-negative human donor milk fed pigs did not affect neurite outgrowth, while all 5 CSF samples (100%) derived from formula-fed NEC-positive pigs induced neurite outgrowth, which was detected after 24 h of CSF sample treatment.

## CONCLUSIONS

CSF collected from preterm newborns may affect neurite outgrowth. Differential effects may relate to diet-dependent colon NEC lesions that via bacterial toxins and systemic inflammatory mediators may reach the CSF and the developing brain. NEC may trigger adaptation mechanisms



in the immature brain by release of neurotrophic and/or anti-inflammatory factors that stimulate neurite outgrowth. The bioactive compounds in CSF and blood that stimulate neurite outgrowth will be studied in more detail.

### ABS 30

#### URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) AFTER GLOBAL HYPOXIA-ISCHEMIA IN NEWBORN PIGLETS

H.T. Garberg, M.U. Huun, G. Dyrhaug, R. Solberg, O.D. Saugstad

*Department of Pediatric Research, Women and Children's Division & Institute for Surgical research, Oslo University Hospital Rikshospitalet, Oslo, Norway*

#### INTRODUCTION

The incidence of acute kidney injury (AKI) after neonatal hypoxia-ischemia is high. AKI is independently associated with the presence of hypoxic-ischemic lesions on postcooling brain MRI. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is a promising marker of hypoxic-ischemic renal injury and shows excellent ability to predict AKI. Cannabidiol (CBD) is a promising neuroprotective agent that is currently being investigated for use after neonatal hypoxia-ischemia. We wanted to assess how global hypoxia ischemia affect urinary NGAL levels in piglets and look for possible nephroprotective effects of CBD, alone and in conjunction with hypothermia (H), as measured by reduced NGAL levels.

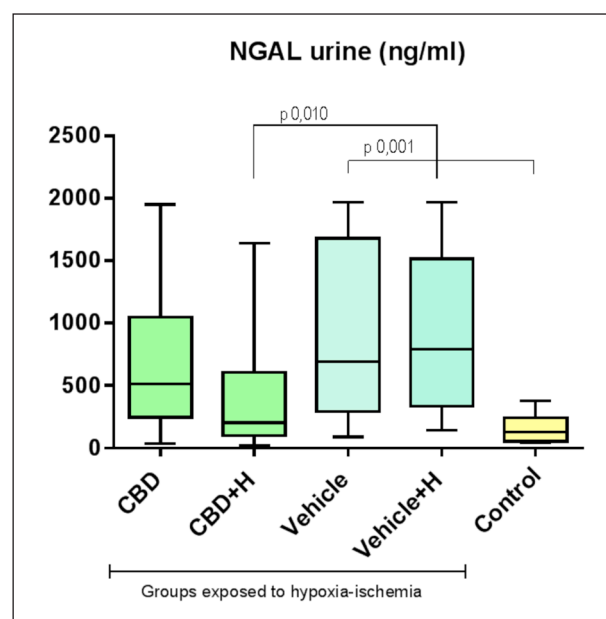
#### PATIENTS AND METHODS

54 anesthetized piglets (age 12-36 hours) were randomized to either undergo global hypoxia (n = 48) until base excess reached -20 mmol/L or mean arterial blood pressure dropped below 20 mm Hg, or to the control group (n = 6). After hypoxia piglets were randomized to the different study groups (each n = 12): CBD (1 mg/kg), CBD (1 mg/kg) + H, Vehicle or Vehicle + H. Urine was collected 9.5 hours after end of hypoxia and snap frozen for further analysis. NGAL levels were measured using a porcine-specific ELISA (Kit-044; Bioporte).

#### RESULTS

Urinary NGAL levels were significantly elevated in asphyxiated piglets compared to control group.

In piglets treated with CBD + H, NGAL levels were comparable to controls and significantly lower than Vehicle + H (Fig. 1).



**Figure 1 (ABS 30).** Urinary neutrophil gelatinase-associated lipocalin (NGAL) in the different groups exposed to hypoxia-ischemia and in control group.

CBD: cannabidiol; H: hypothermia.

#### CONCLUSIONS

Urinary NGAL is a promising, non-invasive, marker of renal injury after neonatal hypoxia-ischemia. Levels are significantly elevated after global hypoxia-ischemia in our piglets. CBD in conjunction with therapeutic H might exert a synergic nephroprotective effect.

### ABS 31

#### CEREBRAL DEEP GREY MATTER ALKALOSIS IN BABIES WITH NEONATAL ENCEPHALOPATHY IS ASSOCIATED WITH AN INCREASED SEIZURE BURDEN

C. Uria-Avellanal<sup>1</sup>, D. Price<sup>2</sup>, M. Sokolska<sup>2</sup>, S. Mitra<sup>1</sup>, A. Bainbridge<sup>2</sup>, X. Golay<sup>3</sup>, N. Robertson<sup>1</sup>

<sup>1</sup>Neonatal Department, UCL Institute for Women's Health and UCL Hospital, London, UK

<sup>2</sup>Medical Physics and Bioengineering Department, UCL Hospital, London, UK

<sup>3</sup>MR Neurophysics and Translational Neuroscience, UCL Institute of Neurology, London, UK

## INTRODUCTION

Neonatal encephalopathy (NE) following hypoxia-ischaemia happens in 1-2/1,000 newborn babies in the UK, causing death or severe disability in over 50% of them despite receiving therapeutic hypothermia. Phosphorus spectroscopy (31P MRS) provides unique information on brain intracellular pH (pHi). Alkalosis has been linked with poor outcome in the pre-cooling era (Robertson et al., 2002), and more recently it has been associated with increased seizure burden in a rodent model (Helmy et al., 2011).

We hypothesised that deep grey matter pHi would be a reliable biomarker of brain injury in babies with NE who underwent cooling, and the degree of alkalosis would correlate with seizure burden.

## PATIENTS AND METHODS

Ethical approval and informed consent were obtained before the study. We included 16 babies at risk of brain injury in the study, of which 12 were term babies who fulfilled the criteria for therapeutic hypothermia. We excluded from the analysis one of them whose final diagnosis on the MRI was a severe venous infarct. We recorded clinical data, electroencephalographic data (background CFM patterns and seizure burden in minutes over the first 84 hours of life), MRI data (brain injury severity using the Barkovich score for basal ganglia/thalami and for white matter), thalamic proton MRS data (calculating Lactate/N-acetyl-aspartate [Lac/NAA] peak area ratio) and deep grey matter 31P MRS data (measuring energy metabolites and calculating pHi, using the Henderson-Hasselbalch equation).

## RESULTS

Of the 11 babies included in the analysis, mean gestational age was 40<sup>+3</sup> weeks (range: 38<sup>+2</sup>-41<sup>+6</sup>) and mean birth weight 3.46 kg (range: 2.90-4.91). 7/11 were male. They all had an MRI performed between 3-8 days of life (mean  $\pm$  SD: 5  $\pm$  1.4 days of age). 3/11 presented with seizures (seizure burden range: 0-74.1 min).

We found that brain pHi is positively associated with a longer seizure burden ( $p = 0.004$ ).

We also found that brain pHi positively correlates with thalamic Lac/NAA ratio, which is the current biomarker of outcome ( $p = 0.039$ ). All infants with a thalamic Lac/NAA ratio  $> 0.3$  – who are more likely to have a poor outcome – have a deep grey matter pHi above 7.15 (range: 7.16-7.37), similar to previous findings by Robertson et al in whole brain pH in non-cooled babies. There is a negative non-statistically significant trend between other

31P MRS derived energy metabolite ratios and thalamic Lac/NAA.

## CONCLUSIONS

Our preliminary analysis reveals that the degree of brain alkalosis in NE is associated with seizure burden – first time to be described in babies with NE, cooled. This novel finding could be a significant step forward for identification of future neuroprotective therapies and treatment for neonatal seizures in babies with NE, aiming to avoid a rebound alkalosis. More patients are being recruited into the study and outcome data will be analysed.

## ABS 32

### THE PROGNOSTIC VALUE OF NIRS DURING THERAPEUTIC HYPOTHERMIA IN TERM ASPHYXIATED NEWBORNS

P. Costa, A. Graça, I. Sampaio, C. Moniz

*Neonatology Department, Hospital de Santa Maria, Lisbon, Portugal*

## INTRODUCTION

Outcome in newborns with hypoxic-ischemic encephalopathy (HIE) treated with hypothermia is being predicted by amplitude-integrated electroencephalogram (aEEG) and magnetic resonance imaging (MRI). Prognostic role of regional cerebral oxygen saturation (rScO<sub>2</sub>) measured by near-infrared spectroscopy (NIRS) is controversial. We aimed to assess the prognostic value of NIRS in short term outcome prediction in newborns with HIE treated with hypothermia.

## PATIENTS AND METHODS

Term newborns with HIE treated with hypothermia at our NICU were prospectively studied. Newborns were monitored with both NIRS and aEEG during treatment. INVOS 5100 with neonatal sensor and Olympic 6000 monitors were used during treatment and MRI on the second week was performed for outcome prediction. Values of rScO<sub>2</sub> during hypothermia (12, 24, 48 and 72 hours) and the rewarming period (34.5°C, 35.5°C, 36.5°C) were analysed. We categorized monitored patients into three predicted outcome groups (normal, intermediate, adverse) according to both aEEG pattern at 48 hours and MRI.

## RESULTS

In 57 monitored patients, 7 died before MRI and 2 with congenital malformations were excluded. We studied 48 newborns who survived to MRI and predicted outcome was: favorable in 21

**Table 1 (ABS 32).** Regional cerebral oxygen saturation (rScO<sub>2</sub>) values during hypothermia and after rewarming.

		Normal outcome rScO <sub>2</sub> (mean ± SD)	Intermediate outcome rScO <sub>2</sub> (mean ± SD)	Adverse outcome rScO <sub>2</sub> (mean ± SD)
Hypothermia	12 hours	77 ± 7%	80 ± 9%	81 ± 12%
	24 hours	82 ± 8%	82 ± 10%	87 ± 9%
	48 hours	83 ± 7% <sup>a</sup>	87 ± 10%	90 ± 6% <sup>a</sup>
	72 hours	80 ± 7% <sup>b</sup>	79 ± 11%	89 ± 7% <sup>b</sup>
Rewarming	34.5°C	78 ± 7% <sup>c</sup>	82 ± 13%	90 ± 6% <sup>c</sup>
	35.5°C	76 ± 6% <sup>c</sup>	78 ± 13%	89 ± 6% <sup>c</sup>
	36.5°C	76 ± 6% <sup>c</sup>	79 ± 12%	88 ± 7% <sup>c</sup>

<sup>a</sup>p < 0.02; <sup>b</sup>p < 0.01; <sup>c</sup>p < 0.001

(44%), intermediate in 14 (29%) and adverse in 13 (27%). rScO<sub>2</sub> values were significantly higher in the adverse outcome group vs. normal outcome group at 48 hours, 72 hours and during the rewarming period (**Tab. 1**). A cut-off value of 85% was used for rScO<sub>2</sub> associated with an adverse outcome at 48 and 72 hours. Positive predictive values of rScO<sub>2</sub> were 57% and 58%, whereas negative predictive values were 85% and 81%.

#### CONCLUSIONS

During hypothermia rScO<sub>2</sub> may be an early predictor of favourable short term outcome in newborns with HIE treated with hypothermia.

#### ABS 33

#### EEG DISCONTINUITY PREDICTS CEREBRAL TISSUE INJURY AND ADVERSE NEURODEVELOPMENT IN COOLED NEWBORNS

J. Dunne<sup>1</sup>, D. Wertheim<sup>2</sup>, P. Clarke<sup>3</sup>, O. Kapellou<sup>4</sup>, P. Chisholm<sup>4</sup>, J. Boardman<sup>5</sup>, D. Shah<sup>1,6</sup>

<sup>1</sup>Barts and The London School of Medicine and Dentistry, London, UK

<sup>2</sup>Kingston University, London, UK

<sup>3</sup>Norfolk and Norwich University Hospitals, Norwich, UK

<sup>4</sup>Homerton University Hospital, London, UK

<sup>5</sup>MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

<sup>6</sup>Royal London Hospital, London, UK

#### INTRODUCTION

Prolonged EEG discontinuity has been associated with poor neurodevelopmental outcomes after birth asphyxia but its predictive value in the era of therapeutic hypothermia (TH) is unknown.

#### HYPOTHESIS

In infants undergoing TH for hypoxic-ischemic encephalopathy (HIE) prolonged EEG discontinuity is associated with cerebral tissue injury on MRI and adverse neurodevelopmental outcome.

#### PATIENTS AND METHODS

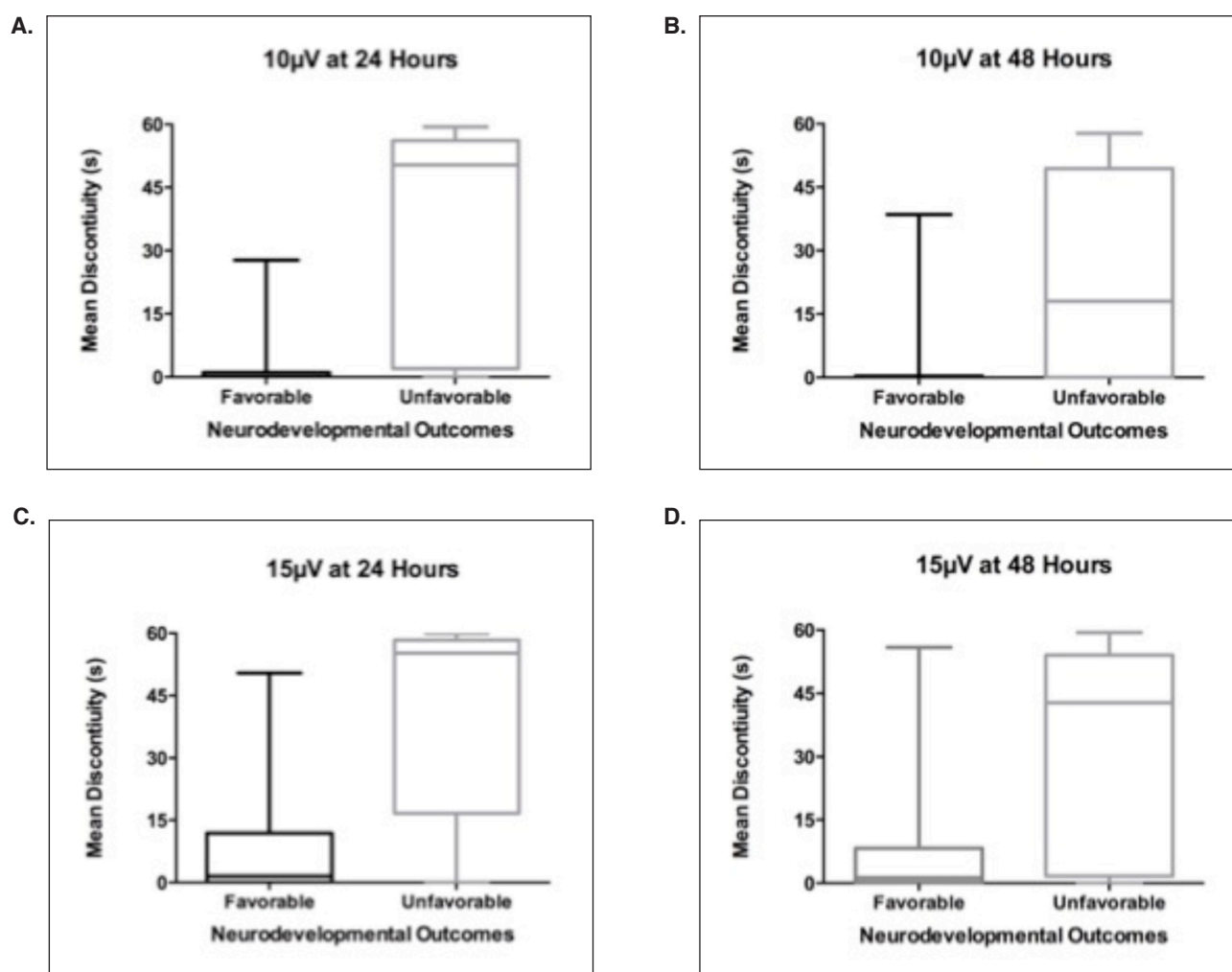
Retrospective study of term neonates from 3 UK centres who received TH for perinatal asphyxia, had continuous 2 channel aEEG with EEG for a minimum of 48 hours, brain MRI within 6 weeks of birth, and neurodevelopmental outcome data at a median age of 24 months. Mean discontinuity was calculated utilizing novel automated software designed for analysis of neonatal EEG recordings.

#### RESULTS

Of 49 eligible infants, 17 (35%) had MR images predictive of death or severe neurodisability (classified as unfavorable outcome). In multivariate logistic regression, mean discontinuity at 24 hours (p = 0.01) and at 48 hours (p = 0.01), high seizure burden (p = 0.05) were associated with severe cerebral tissue injury on MRI. A mean discontinuity > 30 s per minute long epoch, has a specificity and positive predictive value of 100% for unfavorable neurodevelopmental outcome at a 10 µV threshold (**Fig. 1**).

#### CONCLUSIONS

In addition to seizure burden, excessive EEG discontinuity is associated with increased cerebral tissue injury on MRI and is predictive of abnormal neurodevelopmental outcome in infants treated with TH. The high positive predictive value of EEG discontinuity at 24 hours may be valuable in selecting newborns with HIE for adjunctive treatments.



**Figure 1 (ABS 33).** Plots comparing mean discontinuity and neurodevelopmental outcome. The left panels show mean discontinuity at 24 hours and the right panels at 48 hours. The top panels utilise a 10  $\mu$ V threshold and the bottom panels a 15  $\mu$ V threshold. The box edges show the interquartile range and the whiskers the range.

## ABS 34

### A RANDOMIZED CONTROLLED TRIAL OF COOLING COMBINED WITH INHALED XENON FOR PERINATAL ASPHYXIAL ENCEPHALOPATHY WITH CEREBRAL MAGNETIC RESONANCE ENDPOINTS – THE TOBY-Xe TRIAL

D. Azzopardi<sup>1</sup>, N. Robertson<sup>2</sup>, A. Bainbridge<sup>3</sup>, E. Cady<sup>4</sup>, A. Deierl<sup>5</sup>, G. Fagiolo<sup>6</sup>, N. Franks<sup>7</sup>, J. Griffiths<sup>8</sup>, J. Hajnal<sup>1</sup>, E. Juszczak<sup>8</sup>, B. Kapetanakis<sup>1</sup>, L. Linsell<sup>8</sup>, M. Maze<sup>9</sup>, O. Omar<sup>8</sup>, B. Strohm<sup>8</sup>, N. Tusor<sup>1</sup>, A.D. Edwards<sup>1</sup>

<sup>1</sup>Centre for the Developing Brain, Department of Perinatal Imaging, King's College London, London, UK

<sup>2</sup>EGA Institute for Women's Health, University College London, London, UK

<sup>3</sup>UCL Hospitals NHS Foundation Trust, London, UK

<sup>4</sup>Faculty of Engineering Science, University College London, London, UK

<sup>5</sup>Division of Neonatology, Imperial College Healthcare NHS Trust, London, UK

<sup>6</sup>Abide Financial, Bristol, UK

<sup>7</sup>Faculty of Natural Sciences, Department of Life Sciences, Imperial College London, London, UK

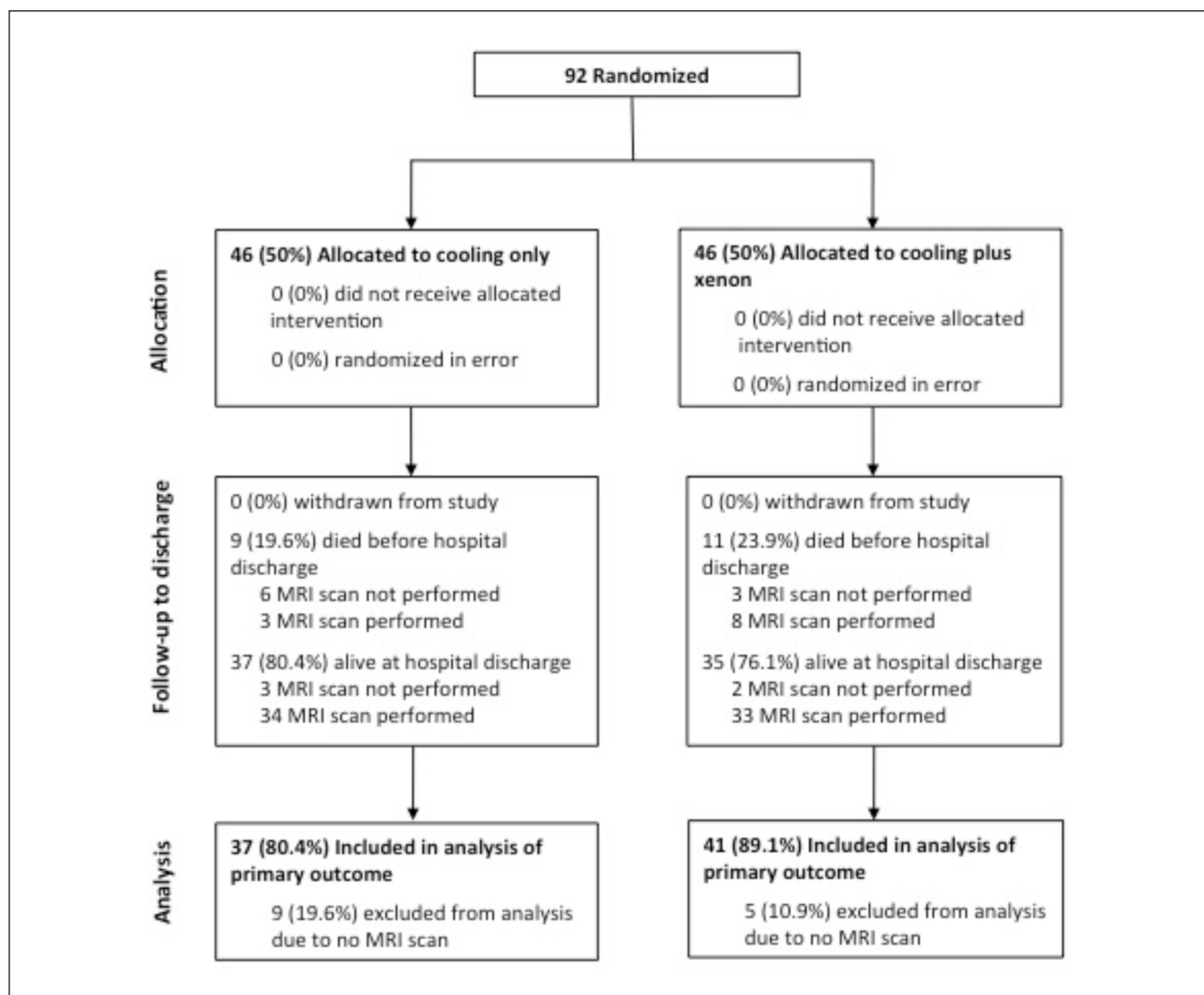
<sup>8</sup>National Perinatal Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK

<sup>9</sup>Anesthesia and Perioperative Care, UCSF School of Medicine, San Francisco, CA, USA

## INTRODUCTION

Moderate cooling following birth asphyxia significantly reduces death and disability, and later outcomes are strongly predicted by cerebral Magnetic Resonance (MR) data obtained soon after birth. Additional therapies may be beneficial, but clinical studies are lacking. We evaluated inhaled xenon gas, a novel therapeutic gas which has





**Figure 1 (ABS 34).** Algorithm in the TOBY-Xe trial.

neuroprotective effects in experimental models, using cerebral MR endpoints.

#### PATIENTS AND METHODS

TOBY-Xe was a multicenter, randomized, two-arm trial. 92 eligible infants of 36-43 weeks gestation were allocated to cooling to a rectal temperature of 33.5°C for 72 hours starting within 6 hours of birth, either alone or combined with 30% inhaled xenon for 24 hours (**Fig. 1**). The main outcomes were reduction in lactate/N-acetyl aspartate (Lac/NAA) ratio in the thalamic region or preserved fractional anisotropy (FA) in the posterior limb of the internal capsule (PLIC) measured within 15 days of birth.

#### RESULTS

Ventilation with xenon commenced at mean (standard deviation) 9.3 (2.5) hours, and continued for 23.4 (3.6) hours. Mean Lac/NAA in the thalamic region and mean FA in the PLIC were similar in the two groups: geometric mean ratio of Lac/NAA, 1.09 (95%

confidence intervals, 0.90-1.32), and mean difference in FA, -0.01 (-0.03-0.02). Clinical outcomes were similar between the two groups. The rates of death were 9/46 (19.6%) in the cooling only group and 11/46 (23.9%) in the cooling plus xenon group.

#### CONCLUSIONS

30% inhaled xenon for 24 hours combined with cooling is feasible and apparently safe, but is unlikely to enhance the neuroprotective effect of cooling following birth asphyxia, when delayed beyond four hours from birth.

Registered at <http://www.clinicaltrials.gov> (NCT00934700).

#### ABS 35

#### MULTIORGAN DYSFUNCTION IN NEWBORNS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN THE HYPOTHERMIA ERA

M. Alsina, A. Martin-Ancel, P. Alamillo, M. Leon, A Garcia-Alix

*Hospital Sant Joan de Déu, Universidad de Barcelona, Barcelona, Spain*

## INTRODUCTION

During the last decade the use of hypothermia in newborns with hypoxic-ischemic encephalopathy (HIE) has become standard clinical practice. However, only scarce data regarding organ dysfunction have been reported in patients treated with hypothermia. The real impact of this therapy in multiorgan dysfunction (MOD) in newborns with HIE has not been evaluated yet.

## OBJECTIVES

(1) To evaluate the frequency and spectrum of severity of MOD in HIE infants in the hypothermia era. (2) To analyze the correlation between organ involvement and the severity of HIE.

## PATIENTS AND METHODS

Consecutive newborns with > 33 weeks of gestation, > 1,800 g of weight at birth, and different stages of HIE, were included prospectively. Severity of HIE was established within the first 6 hours of life, always before starting controlled hypothermia according to a semiquantitative scale. Six organ systems (cardiovascular, renal, respiratory, hematologic, hepatic and internal environment) and 21 biological variables were studied with an asymmetrical grading scale with 3 degrees of involvement: mild, moderate, and severe (they scored 1, 10 and 20 points respectively). This was recorded daily during the 3 first days of life.

## RESULTS

Ninety-one patients were enrolled. All of them presented MOD on day 1. There were differences between the number of organs affected and the severity of HIE ( $p < 0.001$ ). Moreover, 69% of the patients with severe HIE had moderate or severe dysfunction from 3 to 6 organ-systems. The punctuation in the Scale correlated with the stage of HIE as the ROC analysis obtained an area under the curve (AUC) from 0.78 to 0.88 in the 3 days studied. There were significant differences in the severity of involvement of each organ system among the 3 groups of HIE ( $p < 0.05$ ). The most severely affected were respiratory and cardiovascular systems while the most frequently involved were hepatic and internal environment from day 1 to 3.

## CONCLUSIONS

A high correlation between the severity of HIE within the first 6 hours of life and organ injury

during the first 3 days of life was found. Moderate or severe organ dysfunction of more than 2 organ-systems and mainly cardiovascular and respiratory systems were associated with moderate-severe HIE. In this situation, clinicians have to be aware of the neurological exploration and monitoring in infants with perinatal asphyxia.

## ABS 36

### HYPOXIC-ISCHAEMIC BRAIN INJURY: DELIVERY BEFORE INTRAPARTUM EVENTS

D. Odd<sup>1,2</sup>, A. Heep<sup>1,2</sup>, K. Luyt<sup>2</sup>, T. Draycott<sup>1,2</sup>

<sup>1</sup>Neonatal Intensive Care Unit, North Bristol NHS Trust, Southmead Hospital, Bristol, UK

<sup>2</sup>University of Bristol, Bristol, UK

## INTRODUCTION

Hypoxic-ischaemic encephalopathy (HIE) is a major cause of perinatal death and neurodisability. After an infant is born with HIE the question of whether it was predictable, or preventable, is often raised. Perinatal predictors have been well described, however there is little evidence how factors interact. Mothers are increasingly given control over choices around birth, with little robust evidence to inform these choices. The aim of this work is to determine whether a clinical score could be developed to accurately identify women with a higher risk of having an infant with HIE. This is particularly important when elective delivery by CS has been shown to be beneficial in high risk groups.

## PATIENTS AND METHODS

This study is based on the Avon Longitudinal Study of Parents and Children (ALSPAC). This dataset was split into two halves: with each infant being randomly allocated to either cohort 1 or 2. The first cohort was used for the derivation of the model (**Tab. 1**), while it was tested exclusively on the second. Logistic regression modelling was then performed to develop a predictive model. The final model was used to predict the outcome of infants in the second cohort and infants divided into 4 risk quartiles. To give some indication of possible avoidable disease, the proportion of infants with HIE, potentially avoided by earlier delivery, was estimated by assuming that medicalised delivery by elective LSCS at 37 weeks would remove intrapartum risk of HIE for those infants undelivered at this point.

## RESULTS

6,712 infants were randomised to cohort 1. In the final model 7 covariates remained (parity, pre-eclampsia, polyhydramnios, induction of labour, pre-labour rupture of membranes, gender, concerns over fetal growth and prematurity). When applied to the second cohort, a ROC curve (Fig. 1) for the prediction of developing HIE in the newborn period showed good evidence for association (Area Under the Curve of 0.68 [95% CI 0.60 to 0.77]) and the risk score derived was strongly associated with the risk of HIE, resuscitation and stillbirth, and neonatal death (all  $p < 0.05$ ).

Elective delivery of infants in the highest risk 25% of the antenatal population at 37 weeks gestation could prevent 14% of all HIE, with a NNT of 41.

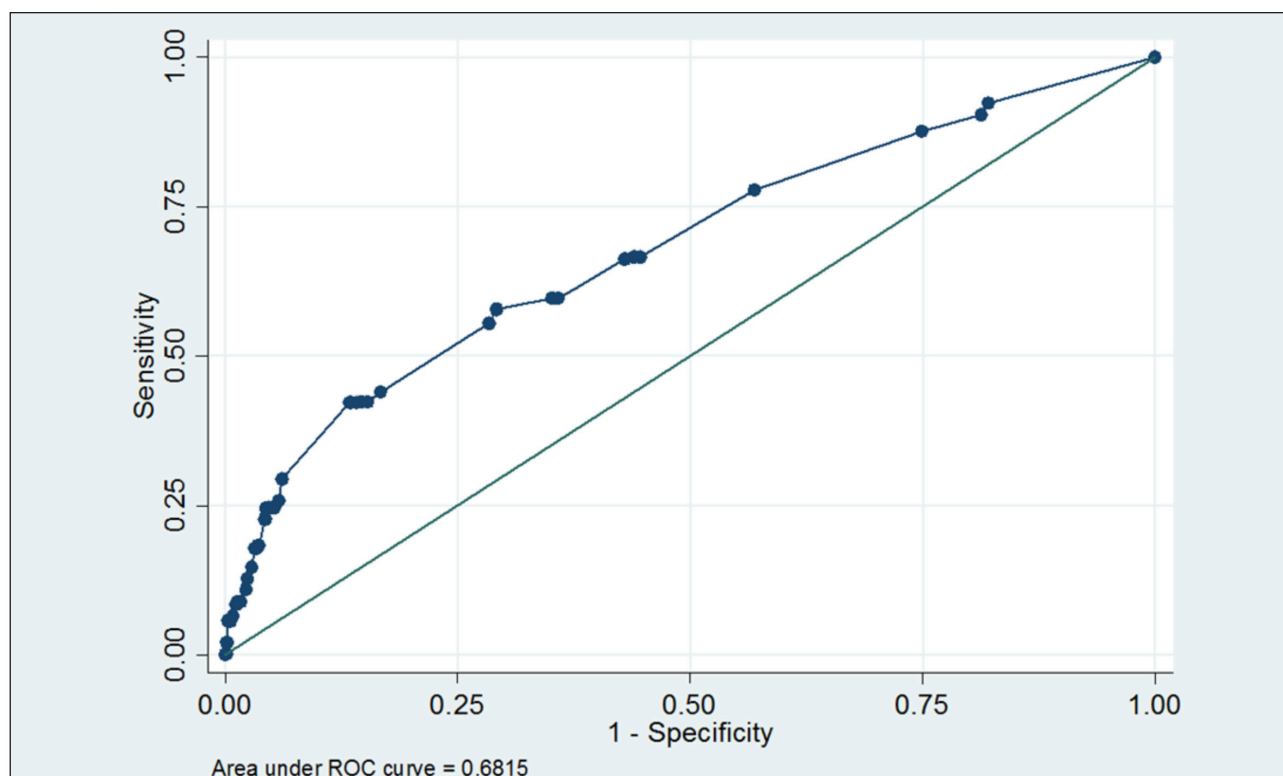
## CONCLUSIONS

It is possible to combine routine antenatal findings to identify infants at higher risk of neonatal HIE, thereby recognising those infants who may benefit most from delivery by elective CS.

This work suggests a clinical risk score permits antenatal identification of high-risk infants whose outcome may be amenable to changes in clinical practice to potentially reduce HIE rates, and its devastating consequences.

**Table 1 (ABS 36).** Full and simplified logistic regression models (cohort 1:  $n = 6,712$ ).

Measure	Saturated model OR (95% CI)	Final model OR (95% CI)
<b>Booking factors</b>		
Maternal age	0.98 (0.92-1.04)	-
Smoking	1.08 (0.58-2.01)	-
Primiparous	1.99 (0.98-4.04)	1.91 (1.01-3.62)
Previous LSCS	0.90 (0.20-4.09)	-
Multiple births	0.99 (0.21-4.68)	-
<b>Antenatal factors</b>		
Pre-eclampsia	4.42 (1.61-12.11)	5.18 (2.02-13.32)
Gestational diabetes	1.13 (0.13-9.85)	-
Threatened abortion	-	-
Placenta previa	4.14 (0.40-43.8)	-
Oligohydramnios	1.29 (0.22-7.54)	-
Polyhydramnios	3.88 (0.79-12.12)	5.03 (1.14-22.30)
Threatened preterm labour	1.51 (0.48-4.76)	-
Male	2.17 (1.14-4.12)	2.34 (1.21-4.52)
<b>Labour factors</b>		
Induction of labour	1.35 (0.68-2.67)	-
Pre-labour rupture of membranes	1.74 (0.86-3.54)	1.82 (0.93-3.59)
IUGR concerns	4.25 (1.64-10.99)	5.28 (2.27-12.28)
Gestation at birth		
Pre-term	1.82 (0.80-4.15)	1.96 (0.91-4.20)
Post-term	1.33 (0.45-3.93)	-
Prelabour breech	1.56 (0.81-2.99)	-
Duration of ruptured membranes (hours)	1.00 (0.99-1.01)	-



**Figure 1 (ABS 36).** ROC of final logistic regression model (cohort 2:  $n = 6,688$ ).

# Neurodevelopmental Outcome

ABS 37

## IS NEONATAL ESTABLISHED HEARING LOSS PERMANENT IN NICU GRADUATES?

J. Kuiper, W. van Huffelen, R. Benard

*Department of Neonatology, Isala Clinics, Zwolle, the Netherlands*

*Pento Audiology Centre, Zwolle, the Netherlands*

### INTRODUCTION

NICU graduates who do not pass Automated Auditory Brainstem Response (AABR) hearing screening at term are tested within 3 months of age by means of an extensive audiology diagnostic program (auditory brainstem response test [ABR], oto-acoustic emissions [OAE], tympanometry and observation audiometry) to diagnose the severity and type of Hearing Loss (HL). The distinguished types of HL are: conductive HL, cochlear HL or auditory neuropathy (AN). The goal of this study is to investigate the predictive value of the initial findings of the best ear at 3 months post-term

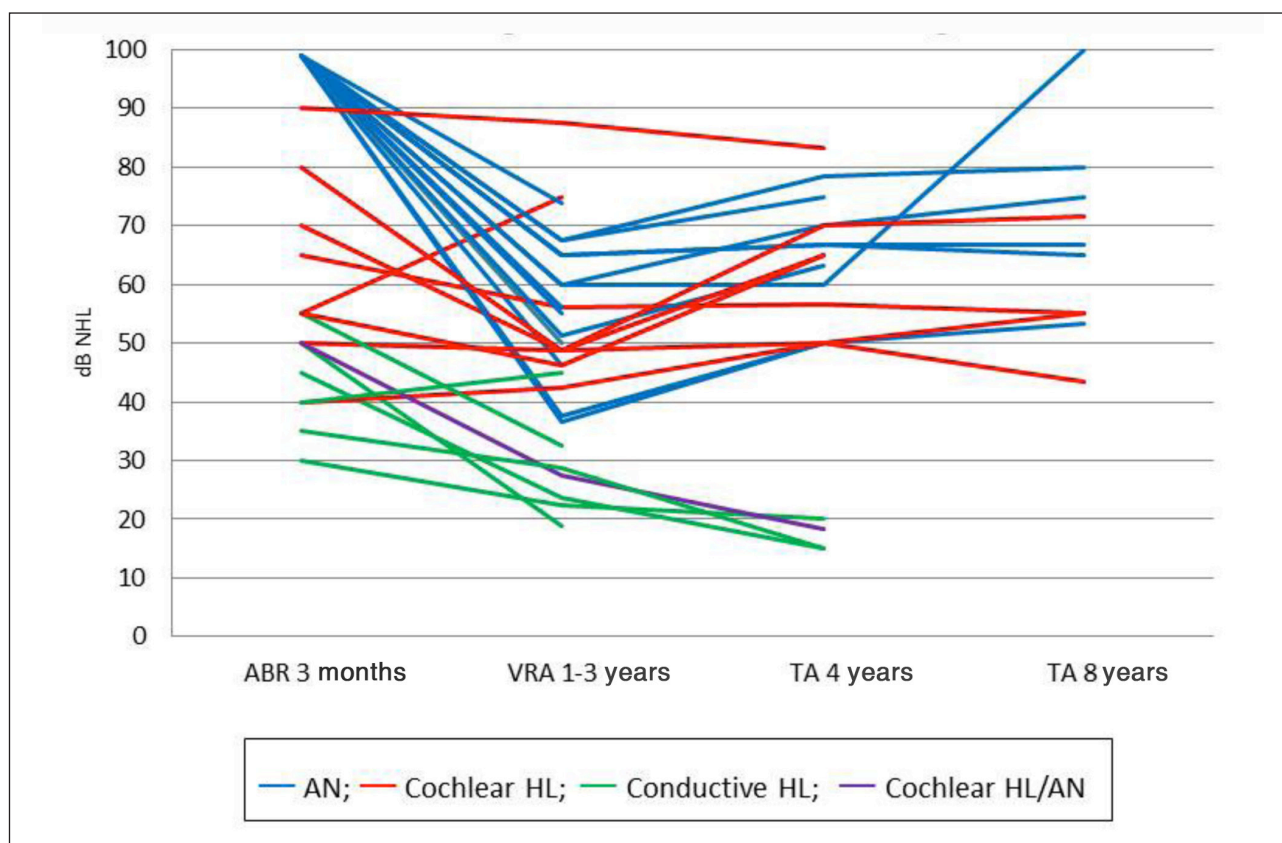
with regard to the type and severity of bilateral HL at 4-8 years.

### PATIENTS AND METHODS

From 2004-2009 62 NICU graduates were referred to the extensive audiology diagnostic program. After diagnostics some children were referred for treatment to other audiology centers and gradually lost to follow-up. Follow-up data of the best ear contains Visual Reinforcement Audiometry (VRA) at 2-3 years, and pure-tone audiometry (average of the frequencies 1, 2 and 4 kHz) at 4-5 years and at 6-8 years of age. AN is considered if no typical ABR response follows at stimuli level > 80 dB, in presence of normal OAE responses.

### RESULTS

At 3 months of age 14/62 (23%) referred NICU graduates had normal hearing, in 2/62 unknown, 2/62 unilateral cochlear HL, 1/62 unilateral conductive and 10/62 (16%) bilateral conductive HL. Thirty three/62 (55%) had bilateral sensorineural HL of whom 10/33 cochlear HL, 19/33 AN, and 1/33 combined unilateral-cochlear and unilateral-AN. In 2/33 the distinction between cochlear/AN was not possible. Follow-up results (**Fig. 1**) showed no change in the type of HL. All children with non-syndromic conductive HL recovered after treatment. Children with unilateral HL



**Figure 1 (ABS 37).** Audiologic follow-up in NICU graduates with congenital bilateral hearing loss.



stayed status quo for 4 years. At the age of 4 and 8 years the initial ABR at 3 months of age was highly predictable in 8/8 children with cochlear HL, in contrast with the VRA at 3 years.

In the AN children at 3 months VRA hearing-levels in 17/17 seems to be better at 3 years, compared to the pure-tone audiometry in 9/10 at 4 years and in 9/9 at 8 years.

## CONCLUSIONS

After a referral at AABR hearing screening in NICU graduates extensive audiology diagnostics 3 months post-term is a good predictor for the type of HL as well as severity of HL in the best ear in case of cochlear HL. In children with AN, VRA thresholds at 3 years is a too positive predictor for the pure-tone audiometry at 4-8 years.

## ABS 38

### SHOULD WE CORRECT FOR GESTATIONAL AGE WHEN ASSESSING DEVELOPMENT IN PRETERM-BORN CHILDREN?

N.H. van Dokkum<sup>1</sup>, J.M. Kerstjens<sup>1</sup>, D.M. van Maanen<sup>1</sup>, A.F. de Winter<sup>2</sup>, A.F. Bos<sup>1</sup>, S.A. Reijneveld<sup>2</sup>

<sup>1</sup>Neonatology Department, Beatrix Children's Hospital, University Medical Center Groningen, Groningen, the Netherlands

<sup>2</sup>Health Sciences Department, University Medical Center Groningen, Groningen, the Netherlands

## INTRODUCTION /CASE REPORT

Development of early preterm-born children (EP, gestational age [GA] < 32 weeks) follows a different pattern compared to fullterms (FT, GA 38-41 weeks), with initial developmental delay and partial catch-up as EP grow older. Therefore, to avoid underestimation of development of EP compared to FT, correction for GA is applied when assessing development of EP during infancy and toddlerhood. Consensus on amount and duration of correction for GA is lacking. The aim of this study was to observe the effect of correction for GA for EP when assessing development.

## PATIENTS AND METHODS

Longitudinal developmental data from a random sample of 310 EP born in 2002-2003, from the prospective community-based cohort study LOLLIPOP were analysed at 3 timepoints (6, 15 and 24 months) with and without correction for GA, and compared to data of 256 FT. Developmental data included 25 milestones within the Dutch version of

the Denver Developmental Screening Test (the Van Wiechen Schema [vWS]), analysing milestones on motor, communication, social interaction and adaptive skills. Percentages of EP and FT passing milestones at 3 timepoints with a timewindow of 4 weeks around the assessment age were compared with chi-square tests with and without correction for GA. In addition, for smiling back and walking unaided exact age of mastering with and without correction for GA was examined.

## RESULTS

Average frequencies of FT passing vWS milestones ranged from 95.5-95.9%. Percentages of EP passing milestones increased when EP grew older, with mean averages unadjusted increasing from 70.5% to 89.2% and adjusted from 88.4% to 90.7%. For EP differences in percentages of children passing milestones adjusted vs. unadjusted at age 6, 15 and 24 months were 19.6%, 8.6% and 1.6% respectively. EP smiled back in response and walked unaided significantly later than FT. After correction EP smiled back earlier than FT, whereas after correction EP still walked later unaided than FT. Results are summarized in **Tab. 1**.

**Table 1 (ABS 38).** Percentages for milestones at 6, 15 and 24 months and median ( $\pm$  SD) in weeks for milestones "smiles back in response" and "walks unaided".

Milestone	Group	Percentages passing milestones
6 months	FT	95.9
	EP unadjusted	70.5 <sup>b</sup>
	EP adjusted	90.1 <sup>d</sup>
15 months	FT	95.9
	EP unadjusted	79.8 <sup>b</sup>
	EP adjusted	88.4 <sup>d</sup>
24 months	FT	95.5
	EP unadjusted	89.2 <sup>a</sup>
	EP adjusted	90.7 <sup>a</sup>
Milestone	Group	Median age in weeks $\pm$ SD
Smiles back in response	FT	5.5 $\pm$ 1.8
	EP unadjusted	12.7 $\pm$ 0.30 <sup>c</sup>
	EP adjusted	<b>2.3 <math>\pm</math> 0.26<sup>c</sup></b>
Walks unaided	FT	60.9 $\pm$ 10.4
	EP unadjusted	74.5 $\pm$ 0.61 <sup>c</sup>
	EP adjusted	63.9 $\pm$ 0.60 <sup>c</sup>

<sup>a</sup>p < 0.05; <sup>b</sup>p < 0.01; <sup>c</sup>p < 0.001; <sup>d</sup>trend: p < 0.10.

Bold and italic printed indicates reaching the milestone significantly earlier.

SD: standard deviation; FT: fullterms; EP: early preterms.

## CONCLUSIONS

Differences in percentages of EP passing developmental milestones with and without correction for GA compared to FT diminished with increasing age. This confirms partial developmental catch up. Our results suggest that correction for GA at and above the age of 2 years may not be necessary.

## ABS 39

### NEUROCOGNITIVE DISABILITIES IN CHILDREN WITH MINOR NEUROLOGICAL DYSFUNCTION BORN VERY PRETERM

T. Kurpershoek<sup>1</sup>, E. Potharst-Sirag<sup>2</sup>, C. Aarnoudse-Moens<sup>2</sup>, A. van Wassenaer-Leemhuis<sup>1</sup>

<sup>1</sup>Department of Neonatology, Emma Children's Hospital, Academic Medical Centre, Amsterdam, the Netherlands

<sup>2</sup>Psychosocial Department, Emma Children's Hospital, Academic Medical Centre, Amsterdam, the Netherlands

## INTRODUCTION

Minor neurological dysfunction (MND) is defined as the occurrence of subtle neurological symptoms in the absence of evident neurological pathology. MND is classified as simple (S)-MND, in case of two or less abnormal clusters of signs and complex (C)-MND, in case of three or more abnormal clusters. At least one quarter to one third of the children born preterm is diagnosed with MND at the age of five. C-MND is related to pre- and perinatal risk factors, and has been associated with learning disabilities, behavioural and motor problems, and psychiatric disorders, whereas S-MND is not. The study objective is to find association between C-MND and neurocognitive disabilities.

## PATIENTS AND METHODS

In a prospective cohort study, 94 children born with a gestational age of less than 30 weeks and/or a birth weight of less than 1,000 grams were assessed at the corrected age of five years. Neurological examination following Touwen, verbal IQ (VIQ), performance IQ (PIQ) and a processing speed quotient (PSQ) using the third, Dutch version of Wechsler Preschool and Primary School Scale of Intelligence, simple reaction time, focused attention and visuomotor coordination measures using the Amsterdam Neuropsychological Tasks, and a working memory measure using Digit Span Task, were administered. Motor skills were evaluated with the Movement Assessment Battery for children (M-ABC-2). The children were classified as 'normal' in case of no or S-MND, 'abnormal' in case of C-MND or non-disabling CP.

## RESULTS

Eighty one percent was classified as 'normal' (49 children without MND, 27 with S-MND), and 19% as 'abnormal' (14 with C-MND and 4 with mild CP [GMFSC 1]). Perinatal and social background characteristics were comparable between groups except for percentage of children being small for gestational age (7.9% in 'normal' group vs. 33.3% in 'abnormal' group [ $p = 0.01$ ]).

The abnormal group had a significantly lower PSQ than the normal group (PSQ 85.5 [SD 15.9] vs. 97.9 [SD 16.4] [ $p = 0.005$ ]), a lower simple reaction time (709 ms vs. 620 ms;  $p = 0.035$ ), and a lower M-ABC percentile score (16.1 vs. 44.7;  $p < .001$ ).

VIQ, PIQ, working memory, focused attention and visuomotor coordination did not differ between S-MND and C-MND (**Tab. 1**). Exclusion of the mild CP cases led to the same results.

**Table 1 (ABS 39).** Comparison of neurocognitive functioning between children born very preterm with normal and abnormal neurological examination.

	Normal neurological examination (n = 76)	Abnormal neurological examination (n = 18)	p-value
<b>Intelligence (WPPSI-III-NL)</b>			
Full scale IQ, mean (SD)	95.6 (15.1)	91.9 (16.6)	0.365
Verbal IQ, mean (SD)	96.5 (15.5)	97.9 (17.7)	0.731
Performance IQ, mean (SD)	95.4 (13.6)	90.9 (15.3)	0.221
Processing speed quotient, mean (SD)	97.9 (16.4)	85.5 (15.9)	< 0.01 <sup>a</sup>
<b>Reaction time (ANT)</b>			
Baseline speed, mean (SD)	620.4 (157.0)	709.2 (160.8)	0.035 <sup>a</sup>
<b>Focused attention (ANT)</b>			
Reaction time, mean (SD)	2,092 (503)	2,275 (584)	0.199
Errors, % of trials (SD)	12.4 (11.2)	10.3 (6.6)	0.334
<b>Visuomotor coordination (ANT)</b>			
Tracking precision, mean (SD)	7.08 (3.8)	9.88 (7.3)	0.140
Tracking stability in mm (SD)	5.3 (3.8)	7.7 (6.1)	0.118
Pursuit precision, mean (SD)	12.1 (4.5)	13.8 (5.7)	0.187
Pursuit stability in mm (SD)	10.7 (7.0)	11.4 (10.2)	0.711
Working memory, no. of correct trials (SD)	6.35 (2.28)	6.28 (1.97)	0.891
<b>Motor development (M-ABC)</b>			
Percentile score, mean (SD)	44.7 (27.5)	16.1 (19.4)	< 0.01 <sup>a</sup>

<sup>a</sup>p-value < 0.05 statistically significant

## CONCLUSIONS

Very preterm infants at the age of five with C-MND have lower Processing Speed Quotient, slower reaction times, and poorer motor skills, than those without MND or with S-MND. Neurological examination remains important in children who were born very preterm. Diagnosing C-MND can help to identify children that need more time to process information.

## ABS 40

### 2 YEAR NEURODEVELOPMENTAL OUTCOMES FOLLOWING EXTREME PREMATURITY OR EXTREMELY LOW BIRTH WEIGHT IN NOTTINGHAM, UNITED KINGDOM. BIRTH COHORT 2000-2008

C. Forster, T. Crosby, J. Dorling

*Neonatal Intensive Care Unit, Queens Medical Centre, Nottingham, UK*

## INTRODUCTION

Many studies have documented improvements in the survival of high risk premature infants, but monitoring the quality of life of these survivors is important as it remains unclear whether improved survival is associated with higher rates of neurodisability. In addition, this data is useful for counselling prospective parents and for comparing unit performance with other similar centres. We report the neurodevelopmental outcomes for babies born between the year 2000 and 2008 in the two tertiary neonatal intensive care units in Nottingham, United Kingdom, who between them deal with 10,000 births and 800 admissions per year.

## PATIENTS AND METHODS

A common dataset comprising antenatal, neonatal and follow-up data was collected on all admitted infants with a birth weight less than 1,001 grams or gestational age less than 31<sup>+0</sup> weeks. Neurodevelopmental assessment at 24 months of age was performed by doctors experienced in developmental follow-up. Outcome was classified according to the Health Status Questionnaire which defines mild, moderate or severe disability and was developed by the National Perinatal Epidemiology Unit and Oxford Regional Health Authority. This has been shown to have good concordance with other criteria for describing disability. If children were not able to attend this appointment, Health Visitors or General Practitioners were asked to complete forms based on routine surveillance information.

## RESULTS

1,423 children were born before 31<sup>+0</sup> weeks gestation or weighing less than 1,001 g. 1,138 survived to 2 years of age (80%), with 220 dying within the first 28 days (77.2% of deaths), a further 29 before discharge (10.2% of deaths), and 36 following discharge (12.6% of deaths).

Outcome data were subsequently available on 977 infants with 120 (10.6%) being lost to follow-up and 41 (3.6%) not entered onto the dataset. 872 (89.3%) had no disability at 2 years of age. 79 (8.1%) had mild to moderate disability and 25 (2.6%) severe disability.

In comparison with national data compiled by the National Neonatal Audit Programme (NNAP), the Nottingham subgroup of 402 babies born before 31<sup>+0</sup> completed weeks of gestation had significantly lower rates of severe disability (2.5% vs. 21.2%,  $p < 0.0001$ ) and significantly higher rates of survival without disability (89.2% vs. 56.3%,  $p < 0.0001$ ).

## CONCLUSIONS

In 2000-2008 high-risk babies cared for in Nottingham had significantly higher rates of survival without disability and significantly lower rates of severe disability. This may reflect good quality perinatal care but it also suggests that improved survival may not always lead to increased rates of disability. An alternative explanation is that the assessments of neurodisability were inaccurate or biased.

## ABS 41

### MONOCHORIONIC DIAMNIOTIC TWINS: A 2-YEAR DEVELOPMENTAL OUTCOME

E. Teixeira<sup>1,2</sup>, M. Machado<sup>1,3</sup>, L.M. Ferreira<sup>1,2</sup>, F. Rodrigues<sup>1,3</sup>, R. Henriques<sup>1</sup>, E. Afonso<sup>1</sup>

<sup>1</sup>Neonatology Department, Maternidade Dr. Daniel de Matos, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

<sup>2</sup>Paediatric Department, Centro Hospitalar Tondela Viseu, Viseu, Portugal

<sup>3</sup>Paediatric Department, Centro Hospitalar Baixo Vouga, Aveiro, Portugal

## INTRODUCTION

Monochorionic diamniotic (MCDA) twins represent only 20% of spontaneous twin gestations but comprise a much higher risk of complications (mainly neurocognitive morbidity) in child and adulthood compared with dichorionic twins and singletons.

Since the rate of twin births has increased over the past few decades along with vastly improved

survival in preterm newborns, a greater emphasis is being placed on achieving good outcomes in long-term survivors.

The purpose of this study was to evaluate neurodevelopmental outcomes in MCDA twins up to 2 years of age.

#### PATIENTS AND METHODS

We performed a retrospective study of all MCDA twins born in a tertiary center from January 2004 to December 2012. Chorionicity was determined by ultrasonographic criteria and confirmed histologically by placenta examination after delivery.

Demographic data, delivery variables, admission in the Neonatal Intensive Care Unit (NICU), perinatal morbidity and mortality and development outcome at 2 years of age were analyzed.

Children were assessed between the ages of 24 and 36 months with Griffiths Mental Development Scales performed by experienced Pediatrician.

Statistical analysis was performed by  $\chi^2$  and t Student tests. A p-value of < 0.05 was considered statistically significant.

#### RESULTS

In this period 260 MCDA twins were born (137 gestations). Several pregnancies were complicated with intrauterine discordant growth (28.5%) and twin-to-twin transfusion syndrome (11.7%). Mean gestational age (GA) was 33.5 weeks and mean birth weight (BW) was 1,957 g. 54.2% were admitted in the NICU: 39.7% needed ventilatory support, 15.6% had anemia, 4.9% had necrotizing enterocolitis, 4.9% had intraventricular hemorrhage and 4.3% had retinopathy of prematurity. Neonatal mortality rate was 3.5%. 88% had a normal neurodevelopmental outcome, 6 children had cerebral palsy, 11 had psychomotor developmental delay, 7 had vision impairment, 4 had hearing loss and 3 had autism. Twins with major neurodevelopmental impairment (11.2%) had a higher incidence of intrauterine growth restriction (38.7% vs. 17.9%,  $p < 0.05$ ), lower mean GA (32.1 vs. 33.9 weeks,  $p < 0.05$ ) and lower mean BW (1,667 vs. 1,997 g,  $p < 0.05$ ).

#### CONCLUSIONS

Similarly to what previous studies have reported, we found that prematurity and lower birth weight neonates have an increased risk of disability at 2 years of age.

Our cerebral palsy prevalence is the same as other studies (2%) and our autism prevalence is the same reported for term infants (1.2%).

All MCDA twins have a high risk of long-term adverse neurodevelopmental outcome and therefore should be followed up regularly into childhood.

## ABS 42

### EPILEPSY IN CHILDREN BORN MODERATELY AND LATE PRETERM

M. Hirvonen<sup>1,2,3</sup>, R. Ojala<sup>1,2</sup>, P. Korhonen<sup>1,2</sup>, P. Haataja<sup>1,2</sup>, K. Eriksson<sup>2,4</sup>, M. Gissler<sup>5</sup>, T. Luukkaala<sup>6,7</sup>, O. Tammela<sup>1,2</sup>

<sup>1</sup>Department of Pediatrics, Tampere University Hospital, Tampere, Finland

<sup>2</sup>Tampere Center for Child Health Research, University of Tampere, Tampere, Finland

<sup>3</sup>Central Finland Health Care District, Jyväskylä, Finland

<sup>4</sup>Department of Pediatric Neurology, Tampere University Hospital, Tampere, Finland

<sup>5</sup>National Institute for Health and Welfare, Helsinki, Finland

<sup>6</sup>Science Center, Pirkanmaa Hospital District, Tampere, Finland

<sup>7</sup>School of Health Sciences, University of Tampere, Tampere, Finland

#### INTRODUCTION

The aim was to compare the incidence of epilepsy in moderately preterm (MP) (32<sup>+0</sup>-33<sup>+6</sup> weeks) and late preterm (LP) (34<sup>+0</sup>-36<sup>+6</sup> weeks) infants to the incidence in very preterm (VP) (less than 32<sup>+0</sup> weeks) and term (T) infants (37 weeks or more) and to identify and compare peri- and neonatal risk factors of epilepsy.

#### PATIENTS AND METHODS

The national register study included all live born infants in Finland in 1991-2008 excluding infants who died before the age of 1 year, had any major congenital anomaly or had missing data. A total of 1,018,256 infants were included in the analysis and they were analyzed in four subgroups (VP, MP, LP and T) and three time periods (1991-1995, 1996-2001 and 2002-2008). Risk factors for epilepsy, related with pregnancy, delivery and mother's and infant's characteristics were sought by Cox's multivariate regression analysis.

#### RESULTS

By the age of 7 years 5,492 children with epilepsy were diagnosed (0.54%). The incidence was 3.0% in the VP, 1.1% in the MP, 0.8% in the LP and 0.5% in the T group. The incidence was lowest in the latest time period in all groups. Intracranial hemorrhage (VP: HR 4.03; 95% CI 2.54-6.38, MP: 5.28; 2.02-13.8, LP: 5.85; 2.13-16.1, T: 3.86; 2.11-7.08) and convulsions (VP: 4.30; 1.50-12.3, MP: 15.5; 1.90-126, LP: 7.60; 2.38-24.2, T: 10.3; 7.80-13.7) were associated to the increased risk of epilepsy in all gestational age groups. Low one-minute Apgar scores predicted an increased risk of epilepsy in all groups, except in the MP group. Compared to the



T group, VP (HR 1.70; 95% CI 1.24-2.34) and LP (HR 1.15; 95% CI 1.01-1.32) births were associated to the increased risk of epilepsy, while MP birth was not. Hyperbilirubinemia was associated with a decreased risk of epilepsy in the VP and MP groups.

## CONCLUSIONS

The incidence of epilepsy decreased by increasing gestational age and by time. Intracranial hemorrhage and neonatal convulsions were strongly associated to the increased risk of epilepsy in all gestational age groups and asphyxia in all, except the MP group. Hyperbilirubinemia seemed to be associated with a decreased risk of epilepsy in the VP and MP groups.

## ABS 43

### SPATIAL LEARNING AND MEMORY IN PRE-TERM PIGS

A.D. Andersen, L. Langhorn, P.T. Sangild, T. Thymann

*Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark*

## INTRODUCTION

Impaired neurodevelopment is a concern following preterm birth. Major functional deficits can be detected early in life but more subtle defects in cognition may not become evident until much later. An animal model to assess cognitive function within the first weeks after preterm birth could help identify supportive interventions. Pig and human brains share similarities in gross anatomical structure and growth spurt in the perinatal period, suggesting that the pig may be a good model to investigate functional brain deficits following preterm birth. We hypothesized that preterm pigs could learn a spatial cognitive task but that learning would be delayed, relative to term pigs.

## PATIENTS AND METHODS

Caesarean-delivered, preterm pigs (n = 17, 90% gestation) from three litters were fed parenteral nutrition for 4 days and increasing volumes of raw bovine milk (32-224 ml/kg/d) until day 23. Beginning on day 15, fasted pigs were tested daily in a spatial T-maze where they learned to navigate via extra maze cues to obtain a milk reward. Pig spatial learning and memory was assessed for six acquisition days (10 trials/day) until reaching the learning criterion (80% correct). This was followed by a 3-day reversal phase in a subsample of pigs

where the previous location of the reward had been reversed. Performance of preterm pigs was compared with that in two age-matched full-term pigs. Pig movements were tracked (EthoVision XT10), providing information on latency to choice and distance moved.

## RESULTS

Initially, pigs performed according to chance (~50% correct choices) and after temporarily showing a response strategy (e.g. always choosing left-turn), the preterm pigs gradually learned to use the visual cues with improved performance over time (p < 0.001) and reached the learning criterion by day 6. Term pigs reached the same criterion after 4 days. Correspondingly, the proportion of correct choices was higher in term vs. preterm pigs (78 ± 6 vs. 64 ± 2%, p < 0.05). Latency to choice and distance moved in the T-maze were similar. During reversal, correct choices were first reduced to ~10% in both preterm and term pigs, but then they improved during further testing although no pigs reached the learning criteria. During this phase, term pigs took longer to make a choice (15 ± 3 vs. 3 ± 0.2 s) and moved a longer distance (245 ± 28 vs. 121 ± 3 cm), relative to preterm pigs (both p < 0.001).

## CONCLUSIONS

Preterm pigs can learn this T-maze task assessing spatial learning and memory. Relative to term pigs, the preterm pigs required more days of training to reach the learning criterion, indicating delayed cognitive development. This test may be useful to investigate effects of dietary or pharmacological interventions on spatial cognition after preterm birth.

## ABS 44

### IS DELAYED NORMALISATION OF PLASMA LACTATE IN COOLED BABIES WITH HYPOXIC-ISCHAEMIC ENCEPHALOPATHY ASSOCIATED WITH A POOR OUTCOME?

N. Ide Bergamaschi<sup>1</sup>, P. Clarke<sup>2</sup>

<sup>1</sup>Norwich Medical School, University of East Anglia, Norwich, UK

<sup>2</sup>Neonatal Intensive Care Unit, Norfolk and Norwich University Hospital, Norwich, UK

## INTRODUCTION

Lactate is produced by anaerobic glucose metabolism during tissue hypoxia and is a commonly measured biomarker in asphyxiated babies. Raised plasma lactate correlates with degree of asphyxiation. Time

to normalisation of plasma lactate has correlated with severity of brain injury in the animal model and with EEG grade and seizure burden in normothermic babies after hypoxic-ischaemic encephalopathy (HIE). Whether lactate levels correlate with later outcomes in cooled HIE babies is unclear. We examined the hypothesis that a delayed time to normalisation of plasma lactate is associated with a poor outcome in encephalopathic term babies who underwent therapeutic hypothermia.

#### PATIENTS AND METHODS

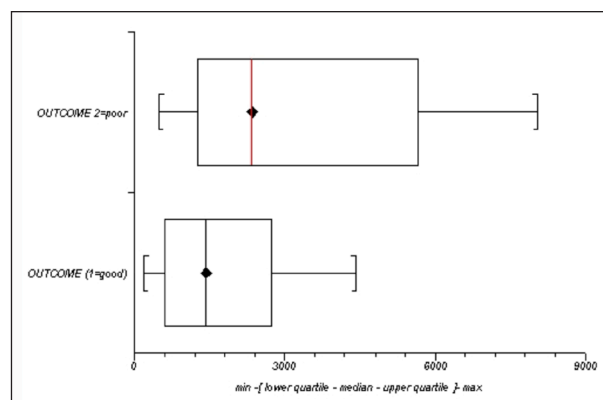
We reviewed clinical records of term/near-term ( $\geq 36$  weeks) babies cooled for HIE (Sarnat grades 1-3) on our NICU in the period Oct 2007-Oct 2011. All lactate levels done between birth and discharge were reviewed to determine the time to normalisation (level  $< 3$  mmol/L) of plasma lactate. Babies were classed according to outcome, with a poor outcome being defined by any one of the following: severe pattern of injury on MR brain scan done within 3 months, severely abnormal 2 year neurodevelopmental outcome on formal testing, or death in infancy. The time duration between birth and lactate normalisation was compared between the two outcome groups using the Mann-Whitney test and a p-value  $< 0.05$  was considered significant. Babies who died before lactate normalisation were excluded from analysis.

#### RESULTS

56 babies fulfilled eligibility criteria within the study period and data were available for 41 (73%) of them. 24/41 (59%) babies underwent a MR brain scan, 9/41 (22%) died before discharge, and neurodevelopmental follow up data were obtained for 30 (94%) of 32 survivors. Overall 23/41 (56%) had a good outcome and 18/41 (44%) had a poor outcome, though 5/41 (12%) died before lactate normalisation without an MR brain scan so had to be excluded from analysis. Median (range) time to lactate normalisation was 23.1 h (2.9-73.5 h) in the 23 babies with good outcome vs. 39.1 h (8.1-134.1 h) in the 13 who survived with a poor outcome (p-value = 0.04, Mann-Whitney U test) (**Fig. 1**).

#### CONCLUSIONS

These data are the first to report the time to normalisation of plasma lactate concentrations in babies who were cooled and to examine associations with later outcomes. In babies with HIE who undergo therapeutic hypothermia, a delayed time to normalisation of plasma lactate is significantly associated with a severe pattern of injury on the MR brain scan in early infancy and/or a severely abnormal neurodevelopmental outcome in infants who survive.



**Figure 1 (ABS 44).** Time to normalisation of plasma lactate levels (mins).

#### ABS 45

#### HEAD GROWTH AND NEURODEVELOPMENTAL OUTCOME AT 1 YEAR FOLLOWING FETAL GROWTH RESTRICTION

M. Boyle<sup>1,2,3</sup>, R. Pinnamaneni<sup>2,4</sup>, J. Unterscheider<sup>2,3</sup>, F. Malone<sup>2,3</sup>, N. McCallion<sup>2,3,4</sup>, A. Foran<sup>2,3,4</sup>

<sup>1</sup>Rosie Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

<sup>2</sup>Rotunda Hospital, Dublin, Ireland

<sup>3</sup>Royal College of Surgeons of Ireland, Dublin, Ireland

<sup>4</sup>Children's University Hospital, Temple Street, Dublin, Ireland

#### INTRODUCTION

Intrauterine growth restriction (IUGR) can be defined as babies whose birth weight lies below the 10<sup>th</sup> percentile for that gestational age and affects up to 10% of all pregnancies. Approximately 5 to 10% of pregnancies complicated by IUGR will result in either stillbirth or neonatal death. The effects of IUGR continue beyond the neonatal period and may have a profound impact on child growth and development. In one study of 1,116 fetuses (PORTO study), abnormal Doppler was significantly associated with adverse obstetric and neonatal outcomes, regardless of estimated fetal weight. We looked at a subgroup of these infants and monitored their head growth and neurodevelopment.

#### PATIENTS AND METHODS

Participants were recruited from the PORTO (Prospective Observational Trial to Optimize paediatric health in IUGR infants). From this cohort, 74 infants were recruited into the StOOPS (Short-term surrogate Outcome Of infants in the PORTO) study.

Informed consent was obtained; background data and birth anthropometry measurements were recorded. All 74 infants had a detailed 3T MRI brain at term corrected gestational age (37-44 weeks). At 1 year corrected age participants parents were asked to complete a 12 month Ages and Stages Questionnaire (ASQ-3), a questionnaire assessing communication, fine motor, gross motor, social-emotional and problem solving abilities. An occipito-frontal circumference (OFC) was measured by the participants General Practitioner at 1 year corrected age.

## RESULTS

Of the 74 infants, 63 had an EFW < 10<sup>th</sup> centile (IUGR) and 11 infants were term infants (controls) whose weight was appropriate for their gestational age (AGA). Of the 63 SGA infants, 34 had abnormal antenatal ultrasound Doppler's and 29 had normal Doppler's. The results of the 12-month ASQ-3 showed that IUGR infants scored lower in communication when compared to term controls (**Tab. 1**). The difference between groups OFC was statistically significant at delivery (p-value < 0.0001) but not at one year of age (p-value = 0.8074, **Fig. 1**)

## CONCLUSIONS

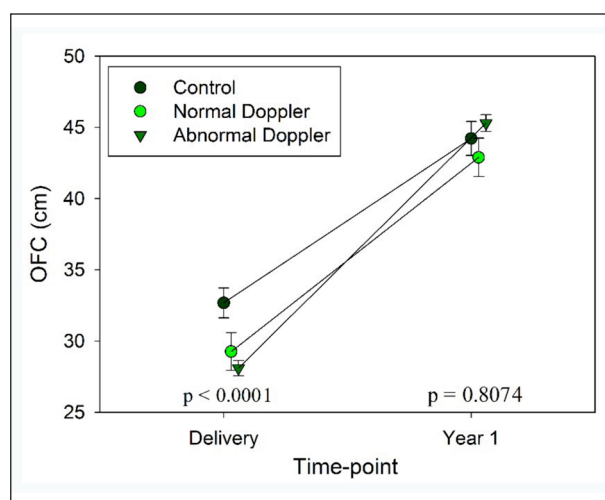
The difference between groups OFC was statistically significant at delivery; the IUGR groups had lower OFC's compared to the AGA term controls; however by 1 year this difference had normalised, displaying catch up head growth. The IUGR infants scored lower in the communication component of the ASQ-3 compared to the controls; this difference was not significant when comparing between the groups with normal and abnormal Dopplers.

**Table 1 (ABS 45).** Comparison of ASQ Scores (Year 1) between groups.

ASQ Item	Control (n = 11)	Normal Doppler (n = 29)	Abnormal Doppler (n = 34)	p-value <sup>a</sup>
Communication	60	47.5	45	0.0109 0.0083 0.8721
Gross motor	40	27.5	30	0.5012 0.5976 0.6855
Fine motor	50	50	50	0.7876 0.7373 0.4712
Problem solving	50	50	45	0.4789 0.1302 0.5450

Medians scores are presented.

<sup>a</sup>Normal Doppler vs. Controls and Abnormal Doppler vs. Controls, Normal vs. Abnormal Doppler, respectively, using the Wilcoxon Rank-Sum test.



**Figure 1 (ABS 45).** Correlation between occipito-frontal circumference (OFC) at delivery and at 1 year of age.

## ABS 46

### EEG SLEEP SLOW WAVE ACTIVITY AS A POTENTIAL MARKER OF LOAD-DEPENDENT DEFICITS IN EXECUTIVE FUNCTIONS IN VERY PRETERM CHILDREN AND ADOLESCENTS

F. Wehrle<sup>1,2</sup>, B. Latal<sup>2</sup>, R. O’Gorman<sup>3</sup>, C. Hagmann<sup>1</sup>, R. Huber<sup>2,4</sup>

<sup>1</sup>Department of Neonatology, University Hospital Zurich, Zurich, Switzerland

<sup>2</sup>Child Development Center, University Children’s Hospital Zurich, Zurich, Switzerland

<sup>3</sup>Center for MR Research, University Children’s Hospital Zurich, Zurich, Switzerland

<sup>4</sup>University Clinic for Child and Adolescent Psychiatry, Zurich, Switzerland

## INTRODUCTION

Many children born very preterm experience difficulties in executive functions, e.g., planning abilities. However, significant deficits often only become evident when the cognitive load is high. Sleep slow wave activity (SWA, 1-4.5 Hz EEG power), the key characteristic of deep non-rapid eye movement (NREM) sleep, has been shown to be locally increased after intense use of a certain cortical region. This study investigated whether sleep SWA represents an electrophysiological marker of load-dependent alterations in brain networks underlying executive functions, i.e., frontal brain areas, and whether this alteration differs between very preterm and term-born children.

## PATIENTS AND METHODS

A group of 38 very preterm (age [M ± SD] 12.9 ± 1.7 years) and 43 (13.1 ± 2.0 years) term-born participants were assessed with a comprehensive battery of executive function measures. The performance differences between the highest and the lowest demand level within each task were calculated and summarized in a composite score to reflect the overall impact of task demands on performance. All-night high-density sleep EEG (128 electrodes) was recorded in all participants. Normalized SWA (dividing SWA at each electrode by the average SWA across all electrodes to investigate local changes rather than global differences), averaged across the first hour of NREM sleep was obtained and correlated with the composite score.

## RESULTS

Sleep efficiency was high in both groups (approximately 90%). The duration and architecture of sleep were not significantly different between the groups ( $p > .41$ ). Looking at all participants, the composite score was positively correlated with SWA in a widespread cluster of 15 electrodes over frontal brain regions ( $r = .31 \pm .06$ ,  $p < .05$ ). Within this cluster, SWA was higher in those participants with better coping abilities than in those with worse coping abilities ( $p < .05$ ). Additionally, comparing the two groups, very preterm participants showed higher SWA compared to term-born participants when task demands were taken into account ( $p < .05$ ).

## CONCLUSIONS

The locally increased SWA in very preterm children compared to similarly performing term-born peers may reflect increased use of neuronal networks underlying executive functions to achieve normal performance. However, if demands are highest, this compensatory mechanism may fail and lead to the observed impaired performance. This study shows that sleep SWA may represent a marker for load-dependent alterations in brain networks known to be involved in executive functioning.

# Pain and stress

ABS 47

## EFFECT OF SHORTER DELAY TIME AFTER SUCROSE ADMINISTRATION TO REDUCE HEEL STICK RELATED PAIN IN NEWBORN INFANTS

N.J. Meesters<sup>1</sup>, M. van Dijk<sup>1,2</sup>, S.H.P. Simons<sup>1</sup>

<sup>1</sup>Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands

<sup>2</sup>Pediatric Surgery, Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands

## INTRODUCTION

Worldwide, sucrose is used as standard of care to relieve pain of skin breaking procedures in neonates admitted to NICUs. Nevertheless, the time needed for sucrose administration to be effective is unknown. Based on only one study (Blass et al., 1994) most protocols dictate that sucrose should be given at least 2 minutes prior to the painful procedure. This delay time may be less feasible in busy units. The aim of this study is to determine if a shorter delay time would affect its effectiveness.

## PATIENTS AND METHODS

We assessed pain with Premature Infant Pain Profile revised (PIPP-R) scores in neonates who underwent a heel stick procedure at our level III NICU. For the first 100 observations the time between sucrose and the heel stick procedure (delay time) was noted. In the next 50 observations the clinical team was instructed to perform the heel stick 120 seconds after the administration of sucrose. PIPP-R scores were assigned immediately after sucrose administration. A multiple regression analysis was performed with PIPP-R as outcome variable and delay time as predictor variable. Postnatal age, non-nutritive sucking, total volume of sucrose, and gender were added as covariates.

## RESULTS

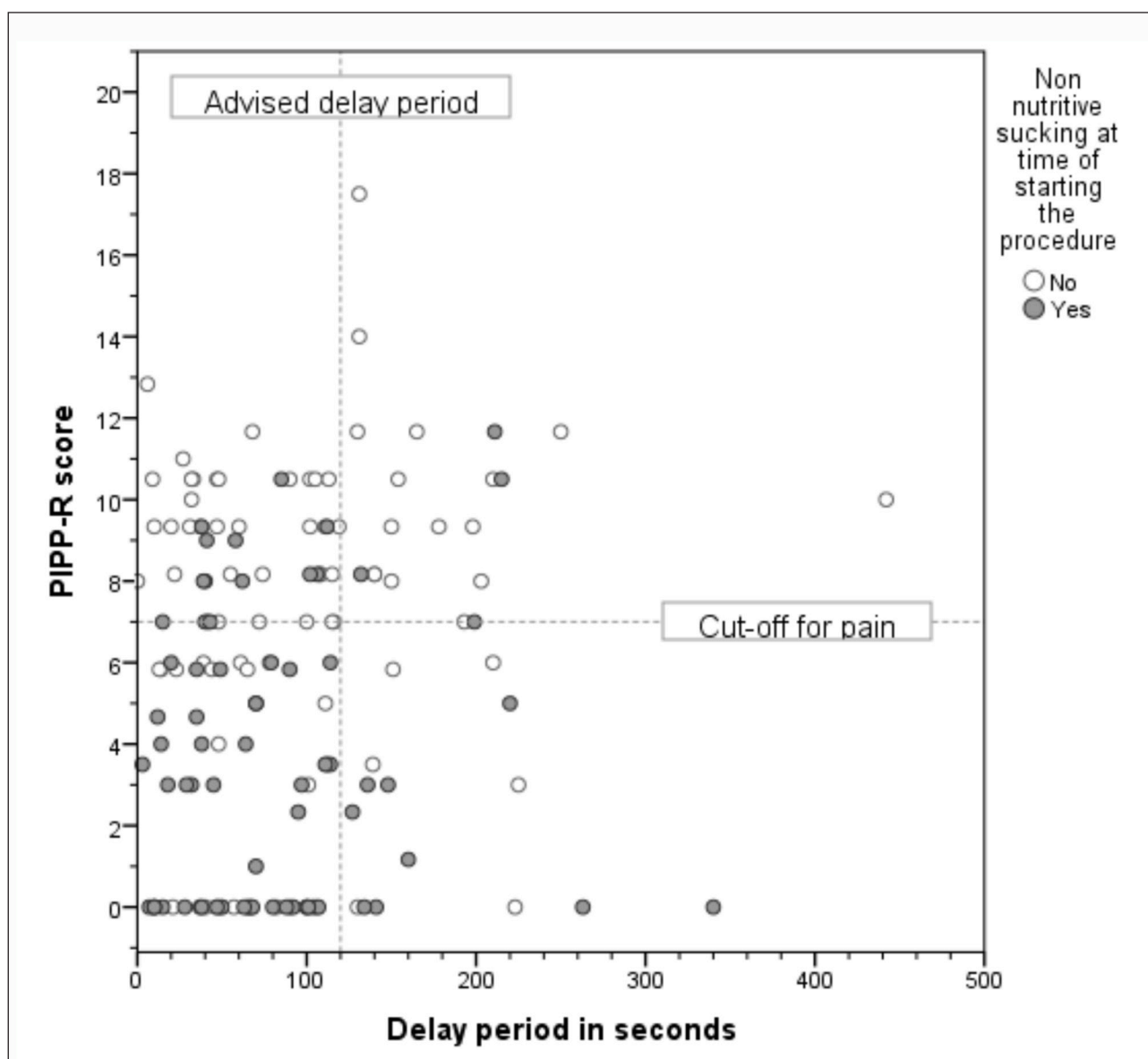
A total of 150 neonates were included with a median gestational age of 30.6 weeks (IQR 27.6 to 33.2 weeks). The median delay time for all 150 observations was 73 seconds (IQR 39 to 115 seconds).

**Fig. 1** shows that there was a large variation in delay time and pain scores. Multiple regression analysis revealed that the delay time had no significant effect on the PIPP-R ( $B = 0.004$ ,  $p = 0.37$ ). Non-nutritive sucking was significantly related to lower PIPP scores ( $B = -3.5$ ,  $p < 0.001$ ).

## CONCLUSIONS

Our study does not confirm the need to wait at least 2 minutes after sucrose has been given. For a busy NICU this may be highly advantageous. As expected, it is best to give sucrose with a pacifier to stimulate non-nutritive sucking.





**Figure 1 (ABS 47).** Large variation in delay period and pain scores in the included neonates.

## ABS 48

### IS MANAGING PAIN A PAIN? NATIONAL SURVEY ON NEONATAL PAIN MANAGEMENT IN THE UNITED KINGDOM

S. Bhayat, H. Gowda

*Luton and Dunstable University Hospital NHS Trust, Luton, UK*

#### INTRODUCTION

Comparing to a few decades ago, it is now well known that newborns, terms and preterms, experience pain. All infants admitted to a neonatal unit will undergo painful procedures. Pain in the neonatal period can have short and long-term effects. Over 40 approved

scales for evaluation of neonatal pain exist. The last survey on neonatal pain done in the UK was done 10 years ago on procedural pain.

#### AIM

To understand if neonatal units in the United Kingdom are evaluating and treating pain in a homogeneous manner, and have neonatal pain management guidelines.

#### PATIENTS AND METHODS

A national survey on pain management was conducted in the UK in April and May 2015. The questionnaire was written by the authors and piloted by a few doctors on the local unit. Units were randomly called, questions were asked by the same person in a standardised manner, the survey was also sent out by email. The questionnaire was aimed at neonatal nurses,

or doctors at any level, and remained anonymous. Data was analyzed by Microsoft® Excel® 2011.

## RESULTS

A total of 36 units responded to the survey. Level 1 unit: 17%, level 2: 36% and level 3: 47%. Only 64% of units have a guideline on neonatal pain management. 69% did not have a pain assessment tool for procedural pain, and 47% for routine pain scoring. It was noted that routine pain scoring was done much less in level 1 (16%) and 2 (46%) neonatal units than in tertiary units (70%). The following non-pharmacological measures for were routinely used for pain relief: sucrose (89%), non nutritive sucking (92%), feeding (67%), skin to skin (67%), cocooning/containment (94%), music (11%). Sucrose was used prior to heel pricks in 67% of units, prior to IV cannulation in 83%, prior to lumbar punctures in 75%. Premedication for endotracheal intubation was used in 83% of units. Amongst the units who are not routinely using pain evaluation scale, 70% would consider making it part of their practice.

## CONCLUSIONS

The survey shows non-homogeneous practice of pain management in the United Kingdom. Despite Cochrane reviews and new studies, many units in the United Kingdom are still not using pain relief such as sucrose and containment, for minor and daily procedures like heel pricks. Music is something very rarely used even though it has been shown to have therapeutic properties.

# Placenta and Prenatal Factors

## ABS 49

### INSULIN-LIKE GROWTH FACTOR-1 PROTEIN CONCENTRATION INCREASES PERINATALLY AND REMAINS HIGH POSTNATALLY IN LAMBS, MIRRORING THE PROFILE IN HUMANS FROM THE FOETUS TO THE ADOLESCENT

M. Dahl<sup>1</sup>, D. Keefe<sup>2</sup>, J.-K. Chung<sup>2</sup>, N. Barton<sup>2</sup>, R. Ward<sup>1</sup>, K. Albertine<sup>1</sup>

<sup>1</sup>Pediatrics, University of Utah, Salt Lake City, UT, USA

<sup>2</sup>Shire, Lexington, MA, USA

## INTRODUCTION /CASE REPORT

Insulin-like growth factor-1 (IGF-1) protein level rises slowly during gestation and more rapidly near

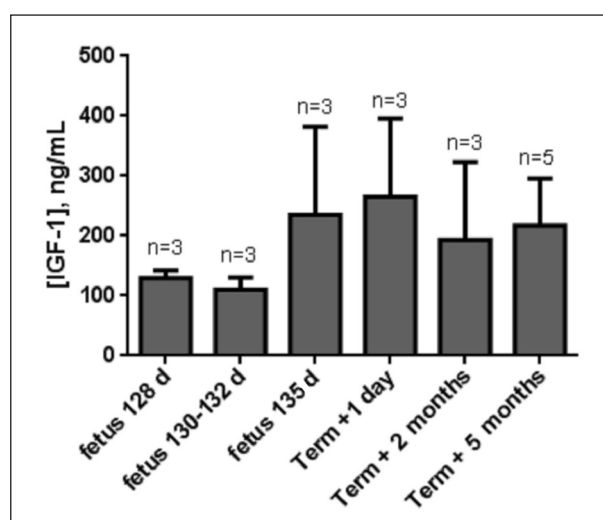
term in preterm human infants and in foetal sheep (from 115 d gestation to near-term). The level doubles perinatally and remains so to adolescence in both species. The level is lower in preterm human infants than in foetuses. Low plasma IGF-1 protein is associated with human chronic lung disease (CLD). We developed a preterm lamb model of neonatal CLD with survival to adolescence. We report initial results for the developmental profile of IGF-1 protein level in foetal, newborn, and adolescent lambs to permit translational study of its relation to CLD.

## PATIENTS AND METHODS

We measured plasma IGF-1 protein level by endpoint ELISA, using a human IGF-1 ELISA kit (Mediagnost; Reutlinger, Germany), the reagents for which cross-react with IGF-1 from many species, including sheep. Serum samples were acid-dissociated prior to analysis to free IGF-1 from binding proteins. IGF-1 level was extrapolated from a standard curve derived from recombinant human IGF-1. Plasma samples were analysed for sheep foetuses at 128 d (~28 wk human), 131 d (~29 wk human), 135 d (~36 wk human), and in lambs after term at 1 d, 2 mo (weaning; ~2 yr human), and 5 mo (adolescence; ~6 yr human). Sample size for this initial analysis (n = 3-5) precluded statistical testing.

## RESULTS

IGF-1 protein level was low in foetal sheep (term 150 d) from 128 d to 131 d. At 135 d, the level doubled and remained so to adolescence (**Fig. 1**). Our results for normal developmental profile of plasma IGF-1 level in sheep are consistent with studies in humans and sheep.



**Figure 1 (ABS 49).** Mean (SD) plasma level of IGF-1 in foetal and postnatal sheep.

## CONCLUSIONS

Plasma IGF-1 level slowly increases in foetal lambs and remains high postnatally to adolescence. Our results provide context for measuring plasma (and lung and brain tissue) levels of IGF-1 protein in preterm lambs with evolving neonatal CLD and former preterm lambs that survive to adolescence. These studies set the stage for treatment to restore IGF-1 to normal plasma values in preterm infants, with evaluation of outcomes for the lung and brain.

## ABS 50

### PRETERM INFANTS BORN WITH PRENATAL ABSENT OR REVERSED END-DIASTOLIC FLOW VELOCITY (AREDV) SHOW SIGNIFICANT LOWER BAYLEY III SCALES AT 2 YEARS – A MATCH CONTROL STUDY

C.N. Chen<sup>1,2</sup>, H.C. Huang<sup>3</sup>, C.Y. Chen<sup>2</sup>, H.C. Chou<sup>2</sup>, W.S. Hsieh<sup>2</sup>, P.N. Tsao<sup>2</sup>

<sup>1</sup>Department of Pediatrics, National Taiwan University Hospital Hsin-Chu branch, Hsin-Chu, Taiwan

<sup>2</sup>Department of Pediatrics, National Taiwan University Children's Hospital, Taipei, Taiwan

<sup>3</sup>Department of Pediatrics, Taipei City Hospital, Taipei, Taiwan

## INTRODUCTION

Prenatal placental insufficiency may significantly contribute to neonatal mortality and morbidity. There are many parameters to evaluate placental function and umbilical artery flow detection is one of the most convincing methods. Fetal Doppler findings with absent or reversed end-diastolic flow velocity (AREDV) are specific poor prognostic factors for neonatal outcome. However, the long-term neurodevelopmental outcome for those preterm infants with AREDV was still controversial. This study aimed to evaluate the neonatal and two-year long-term neurodevelopmental outcomes of those very low birth weight (VLBW) infants with AREDV by Bayley III Scales of Infant Development.

## PATIENTS AND METHODS

We retrospectively collected VLBW preterm infants delivered from January 1, 2011 to December 31, 2012. We specifically identified those infants with AREDV by chart review and clinical characteristics were recorded. Those participants with congenital anomalies, missing data, unclear recorded or loss follow up cases were excluded. Neonatal mortality and morbidity were analyzed and compared between AREDV group and a gestational age (GA)-matched

control group. In addition, neurodevelopmental outcome measured by Bayley III was also be evaluated.

## RESULTS

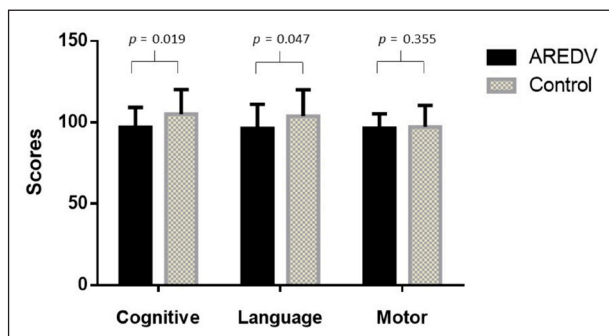
25 AREDV cases survived at least 2 years old and their two-year Bayley III scales were compared to the GA-matched control group with 50 VLBW infants without prenatal AREDV born in the same period (**Tab. 1**). The AREDV group has significantly lower birth body weight, higher incidence of SGA rate and hypoglycemia as compared to the control group. In contrast, the two-year Bayley scales in cognitive and language function were significantly lower in AREDV group as (median [range]) (100 [70-115] vs. 105 [55-140],  $p = 0.019$ ) and (97 [74-127] vs. 106 [65-147],  $p = 0.047$ ) separately, but was not significantly lower in the motor function

**Table 1 (ABS 50).** The clinical characteristics among preterm infants born with prenatal absent or reversed end-diastolic flow velocity (AREDV) in umbilical artery and match control group.

	AREDV (n = 25)	Match control (n = 50)	p-value <sup>a</sup>
<b>GA<sup>b</sup></b>	29 (25-36)	29 (25-36)	0.977
<b>BBW<sup>b</sup></b>	870 (500-1,364)	1,223 (500-1,500)	<b>&lt; 0.001</b>
<b>C/S<sup>c</sup></b>	25 (100%)	40 (80%)	<b>0.016</b>
<b>Male gender<sup>c</sup></b>	15 (60%)	20 (40%)	0.102
<b>Preeclampsia<sup>c</sup></b>	12 (50%)	10 (20%)	<b>0.016</b>
<b>GDM<sup>c</sup></b>	1 (4%)	8 (16%)	0.118
<b>Prenatal medication</b>			
<b>Steroid (&gt; 2 doses)<sup>c</sup></b>	13 (52%)	33 (66%)	0.24
<b>MgSO<sub>4</sub><sup>c</sup></b>	13 (52%)	18 (36%)	0.185
<b>BPD<sup>c</sup></b>	3 (12%)	5 (10%)	0.791
<b>PDA<sup>c</sup></b>	9 (36%)	21 (42%)	0.617
<b>PVL<sup>c</sup></b>	0 (0%)	2 (4%)	0.215
<b>Sepsis<sup>c</sup></b>	3 (12%)	6 (12%)	1
<b>Hypoglycemia<sup>c</sup></b>	7 (28%)	3 (6%)	<b>0.008</b>
<b>SGA<sup>c</sup></b>	22 (88%)	16 (32%)	<b>&lt; 0.001</b>
<b>Bayley III (24 M)</b>			
<b>Cognitive<sup>b</sup></b>	100 (70-115)	105 (55-140)	<b>0.019</b>
<b>&lt; 90<sup>c,d</sup></b>	5 (20%)	3 (6%)	0.064
<b>Language<sup>b</sup></b>	97 (74-127)	106 (65-147)	<b>0.047</b>
<b>&lt; 83<sup>c,d</sup></b>	5 (20%)	4 (8%)	0.132
<b>Motor<sup>b</sup></b>	94 (82-121)	97 (46-130)	0.355
<b>&lt; 94<sup>c,d</sup></b>	7 (28%)	16 (32%)	0.723

<sup>a</sup>Categorical variables by Chi-square test and continuous variables by Mann-Whitney U test; <sup>b</sup>median (min-max); <sup>c</sup>case numbers, n (%); <sup>d</sup>the cut-off points were from Yu YT et al. Res Dev Disabil. 2013;34(11):3875-83.

(94 [82-121] vs. 97 [46-130],  $p = 0.355$ ) (mean  $\pm$  SD in **Fig. 1**). In addition, the AREDV group had more cases with moderate developmental delay in cognitive (20% vs. 6%) and language function (20% vs. 8%) test but not in motor function (28% vs. 32%) as compared to control group.



**Figure 1 (ABS 50).** Bayley III scores at corrected age of 24 month-old. The comparison of Bayley III scales among AREDV and control groups (scores were presented as mean  $\pm$  SD and analyzed by Mann-Whitney U test).

## CONCLUSIONS

The AREDV infants indeed have poor neurodevelopmental outcome in the cognitive, language functions but not in the motor function at 2 years in our study. Early intervention and aggressive physical therapy with rehabilitation program would be recommended for this special population.

## ABS 51

### OCCURRENCE OF ABNORMAL PLACENTAL HISTOLOGY FINDINGS IN TERM ASPHYXIATED INFANTS

A.I. Tóth<sup>1</sup>, M. Szabó<sup>2</sup>, Á. Nándor<sup>3</sup>, E. Széll<sup>2</sup>

<sup>1</sup>Department of Pathology Military Hospital – National Health Center, Budapest, Hungary

<sup>2</sup>1<sup>st</sup> Department of Paediatrics, Semmelweis University, Budapest, Hungary

<sup>3</sup>2<sup>nd</sup> Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary

## INTRODUCTION

Neonatal hypoxic-ischemic encephalopathy (HIE) is still a leading cause of neonatal mortality and long term neurodevelopmental impairment. Histological placental abnormalities may indicate factors contributing to the development

of asphyxia and the subsequent severity of encephalopathy. Our aim was to describe the frequency of histological placental abnormalities in infants suffering from HIE.

## PATIENTS AND METHODS

Conventional placental histology was performed in 34 placentas of newborns diagnosed with HIE and treated with moderate whole body hypothermia between November 2009 and December 2011 at the 1<sup>st</sup> Department of Paediatrics, Semmelweis University. 32 placentas of healthy term newborns were processed as the control group. Chi-squared test and Mann-Whitney's U test were used for statistical analysis.

## RESULTS

HIE vs. control group (mean  $\pm$  SD): GA:  $39 \pm 2$  vs.  $40 \pm 1$  weeks, n.s.; BW:  $3,154 \pm 601$  vs.  $3,575 \pm 469$  g n.s.; 5 min Apgar  $5 \pm 2$  vs.  $10$  ( $p < 0.01$ ). Mortality:  $6/34$  vs.  $0/32$  ( $p < 0.01$ ). Frequency of placental abnormalities in HIE vs. control infants were: chorioamnionitis:  $21/34$  (3 severe, 8 moderate, 10 mild) vs.  $3/32$  (0 severe, 0 moderate, 3 mild) ( $p < 0.001$ ); villous-maturation defect  $12/34$  vs.  $4/32$  ( $p < 0.05$ ); infarcts  $17/34$  (5 acute, 12 chronic) vs.  $5/32$  (4 acute, 1 chronic) (between chronic ones:  $p < 0.001$ ); thrombi  $8/34$  vs.  $8/32$ , n.s.; endangiopathia obliterans  $8/34$  vs.  $4/32$ , n.s.; fetal thrombotic vasculopathy (FTV)  $5/34$  vs.  $0/32$  ( $p < 0.05$ ); villitis of unknown origin  $2/34$  vs.  $0/32$ , n.s.

## CONCLUSIONS

Moderate to severe chorioamnionitis and villous-maturation defect are markedly increased in infants suffering from HIE compared to healthy newborn infants in our cohort. Further studies are needed to answer whether different type of placental pathology results in different pathophysiological subtypes of HIE.

## ABS 52

### THE ANTISECRETORY FACTOR IN PRETERM AND TERM PLACENTAS

A. Gustafsson<sup>1</sup>, E. Fransson<sup>2</sup>, A. Dubicke<sup>3</sup>, S.A. Silfverdal<sup>4</sup>, E. Jennische<sup>5</sup>, S. Lange<sup>6</sup>, K. Bohlin<sup>7</sup>

<sup>1</sup>Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden; Department of Neonatology and Department of Obstetrics, Karolinska University Hospital Huddinge, Stockholm, Sweden

<sup>2</sup>Centre for Health Equity Studies (CHESS), Stockholm University/Karolinska Institutet, Stockholm, Sweden

<sup>3</sup>Department of Woman and Child Health, Karolinska Institute KBH Stockholm, Sweden



<sup>4</sup>Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden

<sup>5</sup>Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

<sup>6</sup>Department of Clinical Bacteriology, Göteborg University, Göteborg, Sweden

<sup>7</sup>Department of Neonatology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden

## INTRODUCTION

Globally, preterm birth (PTB) is the single most important cause of neonatal death. Infants born preterm are at increased risk of morbidity and the consequences of PTB may lead to lifelong disability and high social costs. Inflammation may play a role in the mechanisms leading up to PTB and is also involved in many of the severe complications of prematurity. The pathophysiology of PTB is unclear and effective treatment is lacking. Antisecretory factor (AF) is a protein with a role in the regulation of secretory processes and inflammation. AF may be of importance in the perinatal period. The study objective was to determine AF and inflammatory markers in placenta after term and preterm birth.

## PATIENTS AND METHODS

The material consisted of 61 placenta biopsies, 31 preterm (delivery before 36 completed weeks of gestation) and 30 term (delivery between 37 and 41 completed weeks of gestation). Women  $\geq 18$  years old with spontaneous delivery onset were recruited at the Karolinska University Hospital Solna, Sweden, for a longitudinal project on preterm birth. Women with tobacco use, preeclampsia, diabetes, other systemic disease or fetal malformation were excluded. Placental tissue biopsies were collected after birth and were formalin fixed and paraffin embedded. Immune-histochemical methods were performed to determine AF and inflammatory markers such as CD68. The result was graded into an ordinal scale of seven grades according to the assessment of the immunohistochemistry.

## RESULTS

AF and CD68, the only inflammatory marker reported here, were detectable in all placenta biopsies. Results showed significant differences between AF and CD68 between preterm and term placental tissue ( $p < 0.05$ , Mann-Whitney U test). In preterm placenta there were a low presence of AF and high presence of CD68. In term placentas there were a high presence of AF and low presence of CD68 (**Tab. 1**).

**Table 1 (ABS 52).** Antisecretory factor (AF) and CD68 in placentas after term birth (TB) and preterm birth (PTB).

	Group	n	Mean rank	Sum of ranks
AF, monoclonal 43	TB	30	36.92	1,107.50
	PTB	31	25.27	783.50
	Total	61		
CD68	TB	30	21.67	650.00
	PTB	31	40.03	1,241.00
	Total	61		

## CONCLUSIONS

To our knowledge, this is the first study AF in placenta in relation to preterm birth. The results indicate a link between AF levels in placenta and preterm delivery with an inverse relationship to inflammation. It is a novel finding that may contribute to unravel the pathophysiology of preterm birth and serve as the basis for future research aiming to develop new treatment to prevent preterm birth and its consequences.

## ABS 53

### PRENATAL EXPOSURE TO HYDROXYLATED POLYCHLORINATED BIPHENYLS IS ASSOCIATED WITH MENTAL AND MOTOR DEVELOPMENT OF INFANTS AT THE AGE OF 18 MONTHS

M. Ruel, A. Bos, S. Soechitram, L. Meijer, P. Sauer, S. Berghuis

Department of Pediatrics, Division of Neonatology, University Medical Center Groningen, Groningen, the Netherlands

## INTRODUCTION

Organohalogen compounds, for example polychlorinated biphenyls (PCBs), are widely spread environmental pollutants, known to be neurotoxic for the developing brain. Hydroxylated metabolites of PCBs (OH-PCBs) seem to be even more toxic. However, little knowledge exists on the effects on human health.

## OBJECTIVES

To determine whether prenatal background exposure to OH-PCBs has an effect on the mental and motor development in 18-month-old infants.

## PATIENTS AND METHODS

We included 181 infants of two observational cohorts in the northern part of the Netherlands.

We measured six OH-PCB congeners in maternal blood in the 35<sup>th</sup> week of pregnancy in the first cohort. In the second cohort, we measured three OH-PCBs in maternal blood. We used Bayley's Scale of Infant Development II (BSID-II) to assess infant's mental and motor development at the age of 18 months, the mental development index (MDI) and psychomotor development index (PDI), respectively. We calculated odds ratios with 95% confidence interval (CI) for abnormal scores for each OH-PCB, and adjusted for confounders, i.e. maternal education ( $\geq 14$  years) and gestational age ( $> 40$  weeks).

## RESULTS

The 181 infants had a mean MDI of 97.1 and a mean PDI of 90.1. Twenty-four infants (13%) had MDI  $< 85$ , including 3 infants (2%) with a score  $< 70$ . Seventy-five infants (31%) had a PDI  $< 85$ , including 6 infants (3%) with a score  $< 70$ . We found several associations between prenatal exposure to OH-PCBs and development (**Tab. 1**). After adjustment, higher exposure to 4-OH-PCB-146 (OR = 1.160, CI 1.078-1.249 per 10 pg/g fresh weight (fw);  $p < 0.001$ ) and 4-OH-PCB-187 (OR = 1.080, CI 1.002-1.163 per 10 pg/g fw;  $p = 0.043$ ) was associated with lower scores ( $< 85$ ) on the MDI. Moreover, 4-OH-PCB-107 (OR = 1.453, CI 1.060-1.993 per 10 pg/g fw;  $p = 0.020$ ) measured in one cohort, was also associated with low MDI scores. In the other cohort, exposure to 4-OH-PCB-107 was positively associated with the PDI score (OR = 0.882, CI 0.778-0.999 per 10 ng/g fw;  $p = 0.048$ ).

## CONCLUSIONS

Our findings indicate that prenatal background exposure to OH-PCBs have an adverse effect

on mental development at the age of 18 months, whereas exposure to 4-OH-PCB-107 seems to have a positive effect on motor development.

# Structural and Functional Brain Imaging

## ABS 54

### DEVELOPMENT OF STRUCTURED REPORTING FOR MRI IMAGES OF NEONATES WITH PERINATAL ASPHYXIA

M. Kolossváry<sup>1,2</sup>, A. Lakatos<sup>2</sup>, Z. Bagyura<sup>1</sup>, L.R. Kozák<sup>2</sup>, M. Szabó<sup>3</sup>, P. Maurovich-Horvat<sup>1</sup>, G. Rudas<sup>2</sup>

<sup>1</sup>MTA-SE "Lendület" Cardiovascular Imaging Research Group, Budapest, Hungary

<sup>2</sup>MR Research Center, Semmelweis University, Budapest, Hungary

<sup>3</sup>1<sup>st</sup> Department of Paediatrics, Semmelweis University, Budapest, Hungary

## INTRODUCTION

Daily clinical work and clinical research both require detailed data acquisition and robust data management. The preparation of the clinical report and parallel scientific data collection is time consuming, and increases the probability of mistakes. Therefore, we have developed an innovative online radiology reporting and research platform for MRI images of neonates with perinatal asphyxia.

## PATIENTS AND METHODS

We developed our structured reporting platform in the online PHP based MySQL database system of

**Table 1 (ABS 53).** Odds Ratio for association between abnormal Bayley's Scale of Infant Development (BSID-II) ( $< 85$ ) and OH-PCB-congener levels using multivariate regression analysis.

OH-PCB Congener	BSID-II	OR	95% CI	p-value	Adjusted OR <sup>d</sup>	95% CI	p-value
4-OH-PCB-107 <sup>a</sup>	MDI	0.958	0.852-1.077	0.474	0.958	0.851-1.079	0.481
	PDI	0.957	0.884-1.036	0.276	0.967	0.892-1.049	0.420
4-OH-PCB-107 <sup>a,b</sup>	PDI	0.905	0.812-1.008	0.071	0.882	0.778-0.999	0.048
4-OH-PCB-107 <sup>a,c</sup>	MDI	1.415	1.054-1.900	0.021	1.453	1.060-1.993	0.020
4-OH-PCB-146 <sup>a</sup>	MDI	1.159	1.077-1.247	$< 0.001$	1.160	1.078-1.249	$< 0.001$
4-OH-PCB-146 <sup>a,c</sup>	MDI	1.179	1.070-1.298	0.001	1.194	1.080-1.319	0.001
4-OH-PCB-187 <sup>a</sup>	MDI	1.075	0.999-1.157	0.052	1.080	1.002-1.163	0.043
4-OH-PCB-187 <sup>a,c</sup>	MDI	1.392	1.147-1.689	0.001	1.412	1.155-1.727	0.001

OH-PCB: hydroxylated polychlorinated biphenyls; OR: odds ratio; CI: confidence interval.

<sup>a</sup>Per 10 pg/g fresh weight; <sup>b</sup>RENCO-cohort; <sup>c</sup>GIC-cohort; <sup>d</sup>adjusted for confounders: gestational age and maternal education level.

an online reporting tool. The single and multiple choice questions are structured based on the sequences acquired during the basic protocol for hypoxic ischemic encephalopathy (T1, T2, FLAIR, MRA, DWI). All pathologies seen on any of the sequences were classified using the same conventional anatomical classification in agreement with Radiological Society of North America's radiology reporting initiative MR brain structured report template.

## RESULTS

Overall more than 350 questions were constructed. Using intelligent form visualization only those questions are visible, which are relevant to the initial findings. Furthermore, with the use of default answers, we were able to minimize the burden of questions and maximize the simplicity of the structured reporting form. The structured reporting tool guides the physician through the image analysis and automatically generates a radiological report. Besides the standardized clinical report the system generates a detailed, scientific database, without the need for double data input. The database can be searched, filtered and exported in various formats for further statistical processing.

## CONCLUSIONS

We developed an online radiological reporting tool for MRI images of neonates with perinatal asphyxia. With the use of the online reporting tool the necessity of parallel data acquisition for research and clinical routine can be avoided.

## ABS 55

### THE THERMAL SAFETY OF NEONATAL MAGNETIC RESONANCE BRAIN IMAGING AT 3.0 TESLA

P. Cawley<sup>1</sup>, K. Few<sup>1</sup>, R. Greenwood<sup>2</sup>, P. Malcolm<sup>2</sup>, G. Johnson<sup>3</sup>, P. Lally<sup>4</sup>, S. Thayyil<sup>4</sup>, P. Clarke<sup>1</sup>

<sup>1</sup>Neonatal Intensive Care Unit, Norfolk and Norwich University Hospital, Norwich, UK

<sup>2</sup>Department of Radiology, Norfolk and Norwich University Hospital, Norwich, UK

<sup>3</sup>Norwich Medical School, University of East Anglia, Norwich, UK

<sup>4</sup>Centre for Perinatal Neurosciences, Department of Paediatrics, Imperial College, London, UK

## INTRODUCTION

High field 3.0 Tesla (T) Magnetic resonance (MR) scanners are being increasingly commissioned with the potential for higher quality neuroimaging. Higher radiofrequency energy

and procedural sedation have the potential to alter thermoregulation, especially in post-asphyxial neonates. Overheating is dangerous yet data supporting the thermal safety of 3.0 T MR scanning in neonates are lacking. We therefore aimed to monitor the core temperature in term neonates undergoing 3.0 T MR brain scans. Our hypothesis was that term neonates undergoing 3.0 T MR scans would maintain a core (rectal) temperature within safe homeostatic parameters (36.0-38.0°C).

## PATIENTS AND METHODS

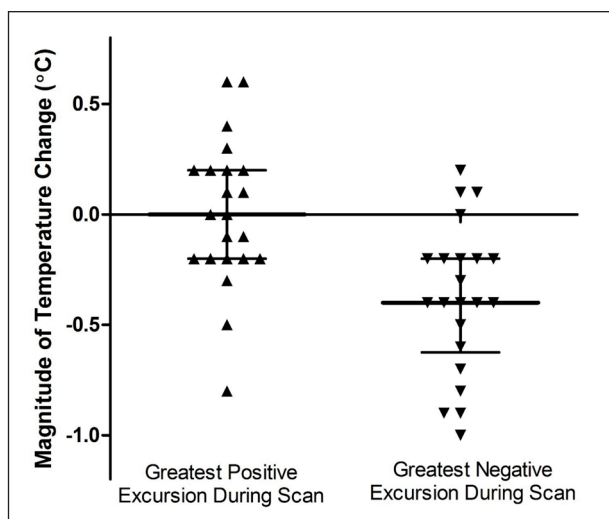
We performed continuous core temperature measurement in consecutively-enrolled term neonates undergoing 3.0 T MR brain imaging as part of the MARBLE study. All neonates had been treated for suspected hypoxic-ischaemic encephalopathy. Rectal thermometry was attained using an MR-conditional fibre-optic temperature system with high-accuracy probe (Linton Instrumentation, UK). MR sequences were performed using the Discovery™ MR750w 3.0T scanner (GE Healthcare, UK). Chloral hydrate was used for sedation. Vital signs were recorded at 5 to 15 minutely intervals. Data were analysed using GraphPad Prism® V5 (GraphPad Software, Inc. CA, USA). Paired pre & post-scan temperatures were compared using Wilcoxon's signed rank test. A p-value < 0.05 (2-tailed) was considered significant.

## RESULTS

Data were obtained from 22 neonates (gestations 37<sup>+6</sup> to 42<sup>+0</sup> weeks). Median postnatal age at scanning was 9 days (range: 5-17 days). Median scan duration was 55 minutes (range: 41-80 minutes). **Fig. 1** shows maximal positive and negative deviations from baseline rectal temperature in individuals during the scan. No significant change in rectal temperature occurred between start and end of the scan (median pre: 36.8°C [IQR 36.7-37.0°C] vs. post: 36.8°C [IQR 36.4-37.1°C], p = 0.16). No infant exceeded a core temperature of 37.5°C during scanning, although the minimum temperature fell to < 36.0°C (nadir: 35.5°C) in 3 (14%) neonates.

## CONCLUSIONS

3.0 Tesla MR brain imaging using the state-of-the-art GE Discovery MR750w scanner does not present a significant thermal challenge to term neonates. These study data are the first to provide reassurance regarding thermal safety in neonates undergoing high-field 3T MR scanning. Our data also suggest that routine use of expensive continuous rectal thermometry may be superfluous in this population.



**Figure 1 (ABS 55).** Deviation of temperature from baseline during MR (all infants).

## ABS 56

### STRUCTURED REPORTING IN HYPOXIC-ISCHEMIC ENCEPHALOPATHY – BENEFICIAL FOR THE RADIOLOGIST AND THE CLINICIAN

A. Lakatos<sup>1,2</sup>, M. Kolossvary<sup>1,4</sup>, M. Szabo<sup>3</sup>, M. Kiss<sup>1</sup>, G. Gyebnar<sup>1</sup>, Z. Bagyura<sup>4</sup>, G. Rudas<sup>1</sup>, L.R. Kozak<sup>1</sup>

<sup>1</sup>MR Research Center, Semmelweis University, Budapest, Hungary

<sup>2</sup>Paediatric Radiology Department, Borsod County Teaching Hospital, Miskolc, Hungary

<sup>3</sup>First Department of Paediatrics, Semmelweis University, Budapest, Hungary

<sup>4</sup>MTA-SE “Lendület” Cardiovascular Imaging Research Group, Budapest, Hungary

## INTRODUCTION

The radiology report is a tool to communicate information to the referring physician and record data for follow-up or research purposes. With structured reporting templates this information becomes uniform, comprehensive and easily manageable. In collaboration with neonatologists and information technologists we developed a structured MRI reporting template for neonatal hypoxic-ischemic encephalopathy (HIE).

## PATIENTS AND METHODS

To enable the development of a systematic reporting template, fifty term neonates with the clinical diagnosis of perinatal asphyxia were enrolled in a retrospective analysis. The selected MRI studies performed with a Philips Achieva 3T MR scanner between 2007 and 2014 were re-evaluated and compared to the clinical

findings and literature data. Based on the clinical question, previous literature, and our own key findings on T1-, T2-, T2\*/SWI-, diffusion-weighted MR images and single voxel MR-spectroscopy an “easy walk through” reporting template was created in a web-based framework. In a feasibility study, we reported 10 neonates through the template as an initial test of the system.

## RESULTS

The proposed structured reporting outline follows a tree structure, it is organized around key structures and key modalities to direct focus on the most characteristic imaging findings seen in neonatal HIE. Although the full reporting template consists of about 350 questions, only the relevant headings and subheadings are to be filled as the report progresses. The first section of the template is composed of patient data and the technical aspects of the MRI examination. The second part records signal intensity changes in 82 nested anatomic landmarks and vascular territories. The central or peripheral pattern of the injury and the possible concomitant findings as hemorrhage or infarction can also be recorded. The feasibility study of 10 neonates showed that the working version of the template provided a precise but also quick and easy way to record the imaging findings.

## CONCLUSIONS

Here we introduced a novel structured reporting template for MRI examinations in HIE. The proposed template is not only useful for the radiologist, but it is beneficial from the clinical, research and administrative point of view. Besides promoting radiologists’ accuracy with a standardized format and expressions, the proposed structured reporting template may reduce interpretation ambiguity for the clinicians, hence it may have a direct positive impact on patient care.

## ABS 57

### DIFFUSE OPTICAL IMAGING OF RESTING STATE FUNCTIONAL CONNECTIVITY IN INFANTS

C.W. Lee<sup>1,2</sup>, R.J. Cooper<sup>1,3</sup>, L.A. Dempsey<sup>1,3</sup>, M. Chalia<sup>1,2</sup>, S. Brigadoi<sup>1,3</sup>, N. Everdell<sup>1,3</sup>, J.C. Hebden<sup>1,3</sup>, T. Austin<sup>1,2</sup>

<sup>1</sup>neoLAB, The Evelyn Perinatal Imaging Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

<sup>2</sup>Department of Neonatology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK



<sup>3</sup>Department of Medical Physics and Biomedical Engineering, University College London, London, UK

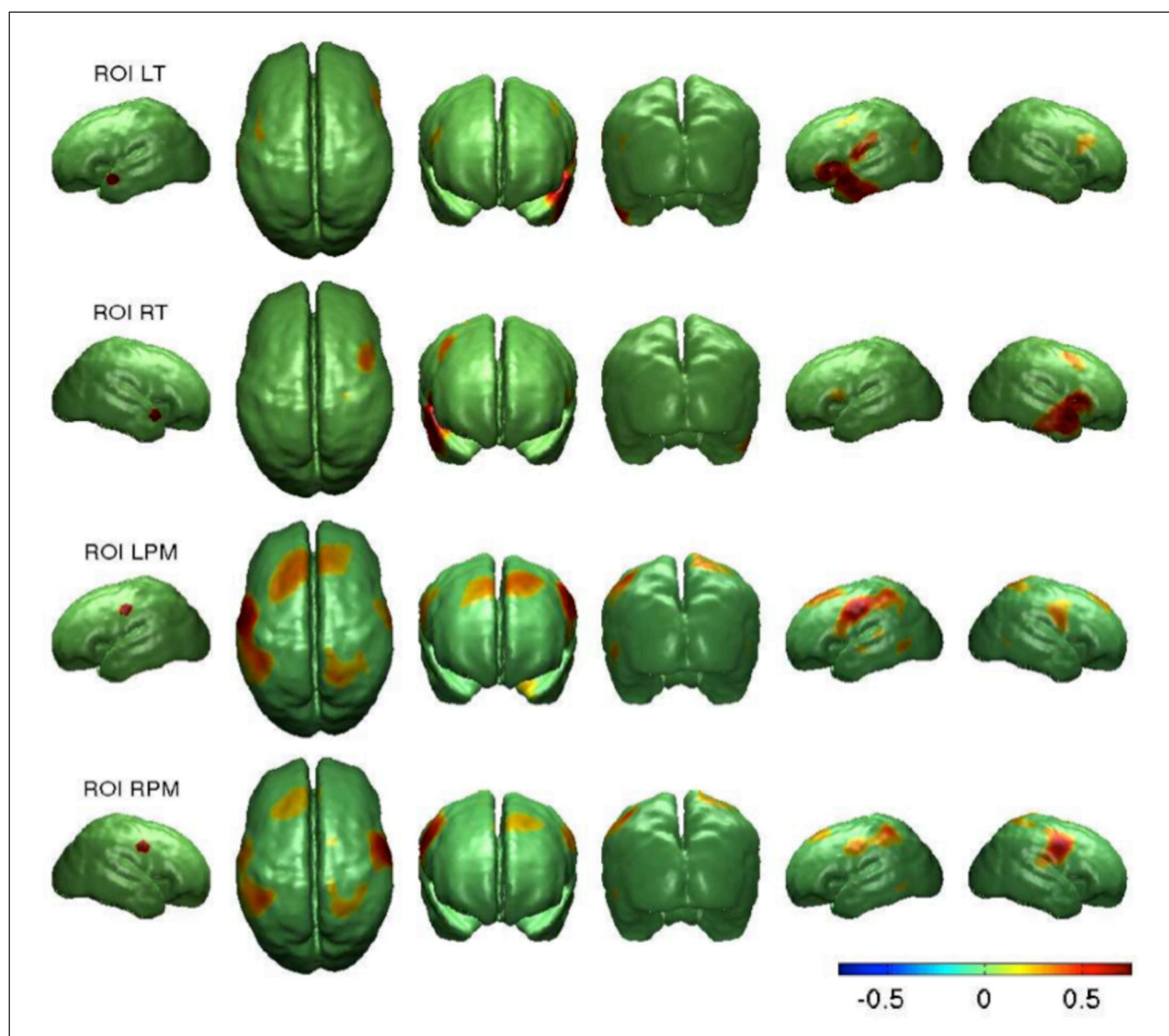
## INTRODUCTION

The growing number of surviving preterm infants has led to an emerging public health problem as these children face lifelong disability. Subtle changes of preterm brain injury can be missed by conventional structural brain-imaging. Functional imaging, such as Diffuse Optical Imaging (DOI) can be used to evaluate spontaneous brain activity known as resting state functional connectivity (RSFC) as a biomarker of brain development. DOI is safe, non-invasive and uses near-infrared light to measure cerebral haemodynamics to

evaluate RSFC images. Identifying atypical development of RSFC at an early stage could facilitate timely neuroprotective strategies to optimise neurodevelopmental potential.

## PATIENTS AND METHODS

The NTS DOI system (Gowerlabs, London, UK) was used to study RSFC in the developing brain. Using a soft, flexible cap (EasyCap, Germany) we have been able to apply a dense array of DOI sensors over the temporal and sensorimotor regions of the head of newborn infants. 19 healthy term infants were recruited for DOI scans. Infants were scanned while asleep for 1 hour in the Evelyn Perinatal Imaging Centre, Rosie Hospital, Cambridge. DOI images of oxyhaemoglobin



**Figure 1 (ABS 57).** Group images from  $n = 15$  subjects. The first column indicates the location of the “region of interest” in red (ROI). Correlated regions are highlighted in orange-red colour along the corresponding row (colour threshold  $r > 0.2$ , colour bar is located at bottom right corner of figure). The image views for each column are: 2<sup>nd</sup> = caudal; 3<sup>rd</sup> = frontal, 4<sup>th</sup> = occipital, 5<sup>th</sup> = left temporal, 6<sup>th</sup> = right temporal. LT: left temporal; RT: right temporal; LPM: left premotor; RPM: right premotor.

concentration changes were reconstructed using an age appropriate neonatal head atlas and a multispectral approach with the TOAST forward modelling and image reconstruction package. Functionally connected brain regions demonstrating correlating slow changes of oxyhaemoglobin were identified to create RSFC image maps.

## RESULTS

DOI images were reconstructed from a total of 15 subjects (median gestational age at birth: 40 weeks) (**Fig. 1**). DOI scans were performed within the first week of life (mean: 2 days). Four subjects were excluded due to motion artifacts in their data. Seed-based analysis and Pearson's cross correlation coefficient  $r$  were used to identify RSFC temporal and premotor networks in the RSFC frequency range (0.009-0.08 Hz). Correlation coefficient  $r$ -values were normalised using the Fisher Z transformation for group analysis. The inverse mean Z-scores produced mean  $r$ -values that were used to create group RSFC image maps. Group analysis revealed images resembling bilateral RSFC networks between homotopic temporal and premotor regions.

## CONCLUSIONS

Our results demonstrate the potential use of DOI as a clinical neuroimaging tool. Our next step is to develop a robust biomarker of brain function by combining DOI RSFC with resting-state EEG and imaging in preterm infants longitudinally to complement clinical assessment of infant neurobehaviour and long-term outcomes.

## ABS 58

### IN VIVO ASSESSMENT OF CEREBRO-CEREBELLAR CONNECTIVITY IN THE DEVELOPING BRAIN USING HIGH ANGULAR RESOLUTION DIFFUSION IMAGING

K. Pieterman<sup>1,2,3</sup>, D. Batalle<sup>1</sup>, J. Dudink<sup>2,3</sup>, D. Tournier<sup>1</sup>, E. Hughes<sup>1</sup>, N. Tusor<sup>1</sup>, A.D. Edwards<sup>1</sup>, F. Hoebeek<sup>4</sup>, S.J. Counsell<sup>1</sup>

<sup>1</sup>Centre for the Developing Brain, Division of Imaging Sciences & Biomedical Engineering, King's College London, London, UK

<sup>2</sup>Erasmus Medical Centre, Division of Neonatology, Erasmus University Rotterdam, Rotterdam, the Netherlands

<sup>3</sup>Erasmus Medical Centre, Division of Radiology, Erasmus University Rotterdam, Rotterdam, the Netherlands

<sup>4</sup>Erasmus Medical Centre, Division of Neuroscience, Erasmus University Rotterdam, Rotterdam, the Netherlands

## INTRODUCTION

The cerebellum grows rapidly during gestation, and is particularly vulnerable during this critical developmental period. The cerebellar hemispheres are connected to contra-lateral cerebral cortices. Abnormalities in these pathways may underpin a range of neurocognitive and neurobehavioral deficits. Delineating these pathways *in vivo*, however, is challenging. The aim of our study was to assess the feasibility of delineating cerebello-thalamo-cortical (CTC) and cortico-ponto-cerebellar CPC pathways during early developmental stages using *in vivo* high angular resolution diffusion imaging (HARDI) analysed with constrained spherical deconvolution (CSD).

## PATIENTS AND METHODS

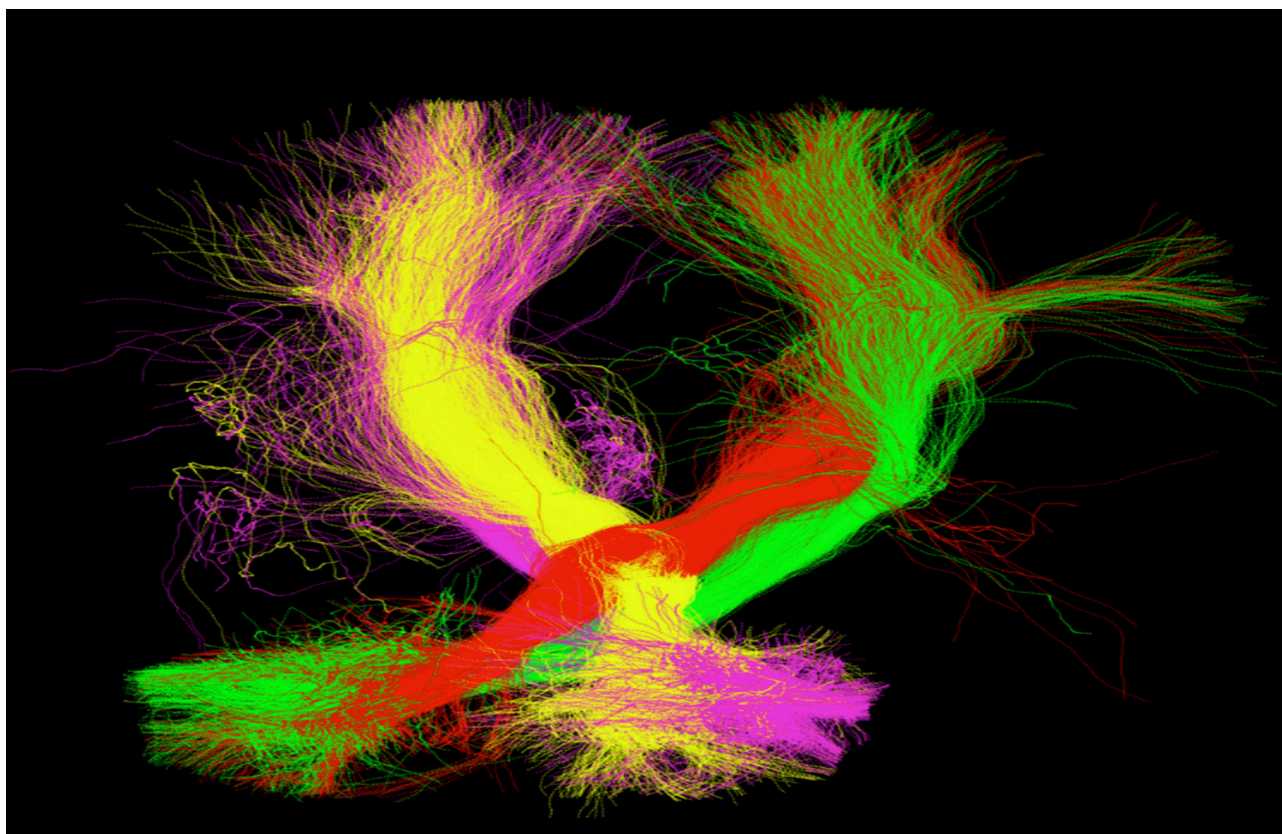
MPRAGE, T2 weighted imaging and HARDI data were acquired in 24 infants on a 3T Philips Achieva MRI system sited on the neonatal intensive care unit. Median (range) gestational age of the infants was 33<sup>+4</sup> (24<sup>+6</sup>-39) weeks. Imaging was performed at a median age of 37<sup>+4</sup> (29<sup>+1</sup>-44) weeks post-menstrual age (PMA). HARDI data was acquired using 64 diffusion-encoding gradients, b-value 2,500 s/mm<sup>2</sup>, and 4 non-diffusion weighted images, voxel size 1.75 x 1.75 x 2 mm. TR and TE were 9,000 and 62 milliseconds respectively. T2 weighted images were parcellated into 90 regions using elastic registration to a neonatal specific atlas. The fibre orientation distribution in each voxel was estimated using CSD and probabilistic tractography was performed using MRtrix.

## RESULTS

Connections between cerebellum and contralateral cerebral hemisphere were identified in all infants studied (**Fig. 1**). Fractional anisotropy (FA) values of CTC and CPC pathways significantly increased with PMA at scan ( $p < 0.001$ ). Regions with the highest percentage of streamlines connecting with the cerebellum through CTC and CPC pathways were: right and left pre-central gyrus, right superior frontal gyrus, right supplementary motor area, right and left postcentral gyrus and paracentral lobule. Regions with highest average FA connectivity were: right and left insula, right caudate and left putamen.

## CONCLUSIONS

Delineating complex cerebello-cortical and cortico-cerebellar fibres and assessing development of these tracts is feasible in the immature brain *in vivo* using HARDI data analysed with CSD. The designed protocol will be useful for assessing the relationship between cerebellar structural development and subsequent cognitive and motor function.



**Figure 1 (ABS 58).** Connections between cerebellum and contralateral cerebral hemisphere.

## ABS 59

### TRIGEMINAL ODOURS RELEASED BY HEALTH CARE PRODUCTS ACTIVATE CORTICAL PAIN PROCESSING AREAS AND TRIGGER PAIN BEHAVIOUR IN PRETERM AND FULL TERM BORN INFANTS

J. Frie<sup>1</sup>, M. Bartocci<sup>1</sup>, H. Lagercrantz<sup>1</sup>, P. Kuhn<sup>1,2</sup>

<sup>1</sup>Karolinska Institute, Astrid Lindgren Children's Hospital, Neonatal Research Unit, Stockholm, Sweden

<sup>2</sup>Médecine et Réanimation Néonatale, University Hospital of Strasbourg, Strasbourg, France

#### INTRODUCTION

Hospitalized newborn infants are highly exposed to nosocomial odorous substances (OS) triggering possibly the intranasal trigeminal subsystem. Irritation of the nasal mucosa can induce pain and activations in pain processing areas in adults. We aimed to measure cortical activation in trigeminal/olfactory and pain areas, to evaluate pain behaviour following OS exposure in newborn infants and to determine whether the amplitude of cortical

activations and intensity of pain behaviour were correlated.

#### PATIENTS AND METHODS

Forty-four newborns (17 full terms, 15 preterms < 33 w and 12 preterms at term age) were included. We used a multichannel NIRS device to record bilaterally cortical activations in the primary somatosensory (S1), and secondary somatosensory cortices (S2) during 50 s (10 s baseline, 10 s presentation, 30 s post-stimuli). We also video recorded the infant's behaviour and two nurses unaware of study purpose performed pain scoring using modified NFCS (0 to 4). Odours were presented in controlled conditions (silent room, active sleep, randomized order) using cotton buds. We presented hand rub in pure solution (HRp) and water as a control. After systematic artefact removal HbO<sub>2</sub> changes from baseline were compared using ANOVA. Means of the maximum NFCS scores for each infant were compared with t-test.

#### RESULTS

In all 44 newborn infants we observed significant cortical haemodynamic changes (HbO<sub>2</sub> increase) in S1 and S2 bilaterally ( $p < 0.001$ ) to HRp but none to water (**Tab. 1**). There was a significant increase in the modified NFCS score during presentation and



**Table 1 (ABS 59).** Significant cortical haemodynamical changes (HbO<sub>2</sub> increase) in S1 and S2 bilaterally.

Location	Hand rub	
	ANOVA	post-hoc <sup>a</sup>
S1 Left	< 0.001	n.s.
S1 Right	< 0.001	0.004
S2 Left	< 0.001	n.s.
S2 Right	< 0.001	n.s.

<sup>a</sup>Post-hoc was calculated using Newman-Keuls.

post presentation ( $1.65 \pm 0.35$   $1.96 \times SE$ ) as compared to baseline baseline ( $0.15 \pm 0.14$   $1.96 \times SE$ ). This increase started during the presentation and lasted during the 30-s post presentation period. The amplitude of the HbO<sub>2</sub> increase to HRp were significantly higher among the infants with maximum NFCS score 2-4 as compared to the ones with maximum NFCS score 0-1 (**Fig. 1**).

**CONCLUSIONS**

Trigeminal OS are perceived by newborns at cortical levels in pain processing areas. HRp

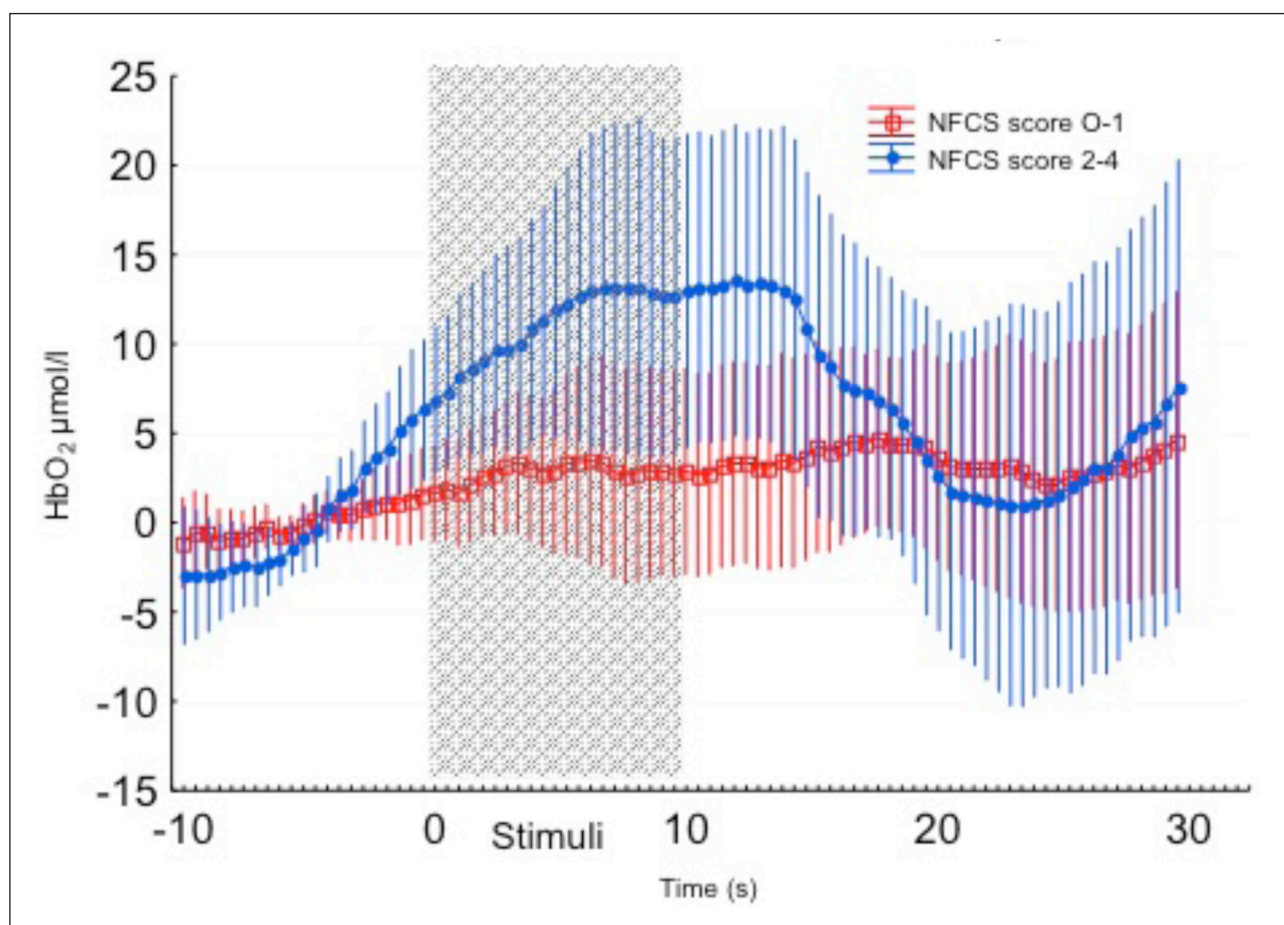
odour can also trigger pain behaviour. Higher amplitude of cortical activation are associated with stronger pain behaviour. These results have implications for clinical practice in order to lessen the exposure of noxious odours to vulnerable infants.

## ORAL COMMUNICATIONS

**ABS 60****PERSISTENT HYPOMETHYLATION OF THE PRETERM PIG INTESTINE**

X. Pan, F. Gao, T. Thymann, P. Sangild

*Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark*

**Figure 1 (ABS 59).** Haemodynamic changes in S1 (left and right) according to pain behaviour.



## INTRODUCTION

Epigenetics play an important role in regulation of tissue-specific gene expression during the perinatal period. It is a molecular mechanism whereby environmental factors transiently or permanently alter gene expression and tissue function. Reduced gestational length, coupled with postnatal maladaptation, induce long-term functional deficits in many organs of preterm neonates (e.g. brain, lungs, intestine). Using preterm pigs as models for preterm infants, we hypothesized that the marked age-, diet and microbiota-related intestinal adaptation in the perinatal period would lead to differences in the intestinal epigenome between preterm and term neonates.

## PATIENTS AND METHODS

After caesarean delivery, preterm and term piglets were kept in environmentally-controlled incubators, and fed identical diets for 26 days after birth (i.e. transition to enteral milk feeding after 5 days of parenteral nutrition). Pigs from both groups were euthanized at birth (before any feeding), on day 5, or after 26 days ( $n = 8-22$ ) and the middle intestine was collected for analyses. Parameters of intestinal structure and function was assessed (mucosal growth, digestive enzymes, permeability, nutrient absorption), and DNA was extracted for a subfraction of the pigs from each group ( $n = 2$ ). Reduced representation bisulfite sequencing (RRBS) was used to assess the genome scale DNA methylation of cytosines at the CpG dinucleotide sites.

## RESULTS

The mid intestine showed marked postnatal increase in mucosal mass in both preterm and term pigs but preterm pigs showed a persistent delay in some digestive enzymes until day 26 (sucrase-isomaltase, maltase-glucoamylase). Compared to the term intestine, the preterm intestine was persistently hypomethylated across the entire genome from birth to 26 days. The reduction in global methylation in preterms increased from birth ( $-3.0\%$ ) to 5 days ( $-4.6\%$ ), but then decreased at 26 days ( $-2.1\%$ ). The coding sequence (CDS) showed the greatest difference among all the genic elements from birth to 5 days, but the smallest difference at 26 days. Comparative analyses revealed 261, 519 and 366 differentially methylated regions (DMRs) between preterm and term pigs at birth, 5 and 26 days, with  $\sim 72\%$  of DMRs located in intergenic regions.

## CONCLUSIONS

The preterm intestine adapts rapidly postnatally and many parameters become similar to those

in the term intestine. Regardless, epigenetic aberrancy of certain genomic regions is maintained and this is consistent with a persistent delay in some intestinal functions. If and when these overall developmental delays are modifiable and associated with epigenetic changes in specific genes, remains to be investigated.

## ABS 61

### EEG BACKGROUND ACTIVITY AND SEIZURE BURDEN ARE ASSOCIATED WITH BRAIN INJURY ON MRI AND NEURODEVELOPMENTAL OUTCOME IN FULL-TERM INFANTS WITH HYPOXIC-ISCHAEMIC ENCEPHALOPATHY

L. Weeke<sup>1</sup>, G. Boylan<sup>2</sup>, R. Pressler<sup>3</sup>, B. Hallberg<sup>4</sup>, F. Groenendaal<sup>1</sup>, L. de Vries<sup>1</sup>

<sup>1</sup>Neonatology Department, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

<sup>2</sup>Irish Centre for Fetal and Neonatal Translational Research, University College Cork, Cork, Ireland

<sup>3</sup>Section of Clinical Neurosciences and Neonatal Unit, University College London, London, UK

<sup>4</sup>Neonatology Department, Karolinska University Hospital, Stockholm, Sweden

## INTRODUCTION

In infants with hypoxic-ischaemic encephalopathy (HIE) the pattern of injury seen on MRI can predict neurodevelopmental dysfunction in later life, as can the extent of EEG abnormalities, especially the background activity. Electrographic seizures have been associated with a worse short-term outcome, defined as more severe brain injury on MRI and clinical seizures with a worse long-term neurodevelopmental outcome. The objective of this study was to investigate the association between EEG features (background activity and seizure burden), pattern of brain injury using MRI and neurodevelopmental outcome in full-term infants with HIE in the era of therapeutic hypothermia.

## PATIENTS AND METHODS

We evaluated 26 full-term infants with HIE, recruited for a multicentre European trial (September 2011-September 2012), who had continuous 8-channel video-EEG monitoring during the first 72 h after birth, an MRI including diffusion weighted imaging, within the first weeks after birth and neurodevelopmental assessment at two years. EEG background activity at 24 h after birth, total electrographic seizure burden,

severity of brain injury on MRI (using a new and an established score [adapted Barkovich score]), were assessed and correlated with outcome at two years using a standardised protocol of neurological examination and the Bayley Scales of Infant Development, third edition.

## RESULTS

EEG was isoelectric in 6 with a poor outcome in all, including death in 3, CP in 2 and moderate motor impairment in 1. Seven had a severely abnormal EEG, but only 1 had severe motor impairment associated with severe white matter injury on MRI. The other 6 had no ( $n = 2$ ) or mild MRI abnormalities and a normal outcome. Twelve had a moderately abnormal EEG, 1 with severe basal ganglia/thalamic (BGT) injury died, 3 had moderate impairment (1 severe watershed, 2 mild white matter/watershed [WM/WS] injury), the other 5 had no ( $n = 4$ ) or mild MRI abnormalities and a normal outcome. All infants with moderate-severe WM/WS or BGT injury had a high seizure burden and an abnormal outcome. There was a significant relation between EEG background activity ( $p = 0.007$  and  $0.006$ , respectively), seizure burden ( $p = 0.04$  and  $0.028$ ), seizure number ( $p < 0.001$  and  $0.001$ ) and both the adapted Barkovich and new score.

## CONCLUSIONS

In infants treated with hypothermia a severely abnormal background activity at 24 h was only associated with an adverse outcome in the presence of severe injury on MRI. A high seizure burden in combination with moderate-severe injury on MRI was also associated with an adverse outcome. However, this association and how seizure burden and severity of brain injury affect each other, needs further elucidation and investigation in a larger cohort.

# Early developmental care

ABS 62

## NEURODEVELOPMENTAL DISABILITIES AND SPECIAL HEALTH CARE NEEDS AT 12 YEARS IN CHILDREN BORN AT < 26 WEEKS' GESTATION AFTER ACTIVE PERINATAL CARE

A. Farooqi<sup>1</sup>, A. Holsti<sup>1</sup>, B. Hägglöf<sup>2</sup>, M. Adamsson<sup>1</sup>, F. Serenius<sup>3</sup>

<sup>1</sup>Institute of Clinical Sciences, Pediatrics, University of Umeå, Umeå, Sweden

<sup>2</sup>Institute of Clinical Sciences, Department of Child & Adolescent Psychiatry, University of Umeå, Umeå, Sweden

<sup>3</sup>Department of Womens' and Childrens' Health, Uppsala University, Uppsala, Sweden

## INTRODUCTION

Proactive care increases the survival of extremely preterm infants (EPT) but there are concerns that improved survival might increase the rate of disabled survivors. The main aim was to examine neurodevelopmental outcomes and special health care needs in 12 year-old EPT children born at 2 tertiary care centers in Sweden adhering to a policy of universal resuscitation of all infants born alive.

## PATIENTS AND METHODS

Of 213 consecutive EPT live births, 140 (66%) survived to discharge home and 6 infants died in the first year of life. Of the survivors, 132 children (98%) were recruited for the study. The outcomes of 132 (98% of all survivors) surviving EPT children born Jan 1992 through Dec 1998 were compared to a matched control of children born at term. Neurosensory impairments (NSI) were assessed by review of pediatric case records, intelligence by WISC-IV, and functional limitations and special health care needs by validated parental interviews (QUICCC).

## RESULTS

At a mean age of 12 years, 132 of 134 eligible EPT children (98%) were assessed. The rates of cerebral palsy, moderate visual impairment, blindness and deafness were 9.1%, 2.3%, 1.5% and 1.5%, respectively among 132 EPT children vs. 0%, 0%, 0% and 0%, respectively among 103 controls. Intellectual impairment  $< -2$  SD but  $> -3$  SD, and  $< -3$  SD was 17.4% and 13.7% respectively in 132 EPT children vs. 4.9% and 0%, respectively among 103 controls. In 132 EPT children either formally assessed or by chart review, the rates of moderate and severe neurodevelopmental disabilities were 18% and 16%, respectively compared with 4.9% and 0% among controls, respectively. See **Tab. 1** for NSIs and **Tab. 2** for overall disability rates. Special health care needs and school performance will be also presented at the meeting.

## CONCLUSIONS

Active perinatal care of deliveries at the limit of viability has not increased disability rates and these are comparable to similar studies that report significantly lower survival rates.

**Table 1 (ABS 62).** Neurosensory impairments (cerebral palsy, visual and hearing impairments) in children born extremely preterm (EPT group) and children born at term (control group).

	EPT n = 132	Control n = 103	p-value for the group difference <sup>a</sup>
<b>Cerebral palsy</b>			
Mild (ambulant without aid)	5 (3.8%)	0	0.069
Moderate (ambulant with aid)	1 (0.8%)	0	1
Severe (nonambulant, wheel-chair dependency)	6 (3.8%)	0	0.036
Any cerebral palsy	12 (9.1%)	0	0.001
<b>Vision</b>			
Moderately impaired <sup>b</sup>	3 (2.3%)	0	0.25
Severe visual impairment/blind <sup>c</sup>	2 (1.5%)	0	0.5
Any vision impairment	5 (3.8%)	0	0.09
<b>Hearing</b>			
Deaf	3 (1.5%)	0	0.5
Impaired hearing corrected with hearing aids	5 (4.5%)	0	0.03
Any hearing impairment	8 (6.1%)	0	0.01
Any neurosensory impairment	22 (16.7%)	0	< 0.001
Major neurosensory impairment <sup>d</sup>	13 (9.8%)	0	0.001

See "Methods" for classification of neurosensory impairments at the mean age of 12 years.

<sup>a</sup>Comparing proportions of EPT children with NSI to the control group with Fishers exact test; <sup>b</sup>registered for regular ophtalmological treatment at low-vision center at the present assessment; <sup>c</sup>according to examination by an ophtalmologist at the present assessment; <sup>d</sup>includes 1 or more of the following: moderate or disabling cerebral palsy, severe visual impairment including visual acuity < 20/200 without glasses in the best eye or those who were registered at low-vision centres, severe auditory impairment in both ears not corrected with hearing aid and hearing loss corrected partially or fully with hearing aid or an implant.

**Table 2 (ABS 62).** Overall disability in children born extremely preterm (EPT group) and children born at term (control group).

Overall disability	EPT n = 132	Control n = 102	Comparison between extremely preterm (< 25 wks) and control subjects	
			Adjusted OR (95% CI) <sup>a</sup>	p-value
Severe disability <sup>b</sup>	21 (15.9%)	0 (0%)		< 0.001
Moderate disability <sup>c</sup>	24 (18.2%)	5 (4.9%)	7.6 (2.2-26.1)	< 0.001
Mild disability <sup>d</sup>	41 (31.1%)	8 (7.8%)	4.8 (2.2-10.5)	< 0.001
No disability	46 (34.8%)	90 (87.4%)	0.06 (0.03-0.13)	< 0.001

Cognitive disability was classified according to control subjects. IQ scores are presented with as ranges of overall scores in each category; these scores are with a standardization mean of 106.6 (15.7) for controls.

<sup>a</sup>Adjusted OR derived from multiple logistic regression analyses (when appropriate) adjusted for composite social risk (single parents, low maternal education and low income), sex and mother country of birth (Nordic vs. non-Nordic); <sup>b</sup>severe disability: disabling cerebral palsy (nonambulant, wheel-chair dependency), severe visual impairment (visual acuity < 20/200 in the best eye or bilateral blindness), profound hearing loss/deafness or FSIQ ≤ 56 (< -3 SD); <sup>c</sup>moderate disability: moderate cerebral palsy (ambulant with aid), auditory impairment requiring bilateral aids or FSIQ 57-72 (-2 to -3 SD); <sup>d</sup>mild disability: mild cerebral palsy (ambulant without aid) or FSIQ 73-88 (-1 to -2 SD) or unilateral blindness with normal vision in the other eye.

## ABS 63

### PARENT AND NURSE PERSPECTIVES ON PARENTAL PARTICIPATION AND SUPPORT IN 11 EUROPEAN NICUs

A. Axelin<sup>1</sup>, S. Raiskila<sup>2</sup>, B. Westrup<sup>3</sup>, B. Silnes Tandberg<sup>4</sup>, L. Lehtonen<sup>5</sup>; SCENE research group

<sup>1</sup>Department of Nursing Science, University of Turku, Turku, Finland

<sup>2</sup>Faculty of Medicine, University of Turku, Turku, Finland

<sup>3</sup>Astrid Lindgren Children's Hospital-Danderyd, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

<sup>4</sup>Department of Pediatrics, Vestre Viken Hospital Trust, Drammen, Norway

<sup>5</sup>Department of Pediatrics, Turku University Hospital, Turku, Finland

## INTRODUCTION

Active parental participation in infant care has shown to be beneficial to infants' growth and development and the psychological wellbeing of the parents. The objective of this study was to describe the differences between the European NICUs in 1) parental participation and the perceived support from the staff and 2) the support nurses give to parents during NICU care.

## PATIENTS AND METHODS

Prospective survey was conducted with families of preterm infants born < 35 weeks (n = 535, eligible) in 11 NICUs in 6 countries. 259 mothers and 216 fathers participated. Parent participation and perceived support was measured using SMS questions covering the following aspects of family centered care: active listening, participation in care, individualized support and information, shared decision-making, mutual trust, and emotional support. Parents received in a random order 1 out of 8 questions every evening during infant hospital stay. Nurses working at the bedside answered corresponding questions, 1 after each shift during a 3-month period. Responses were rated on a 7-point Likert scale (1-7, higher more positive), 0 = parent not in the unit or nurse did not work with parents.

## RESULTS

The mothers rated their level of participation higher than the fathers (5.8 [95%CI 5.7-5.8] vs. 5.6 [95%CI 5.5-5.7], mean difference 0.1 [95%CI 0.05-0.2],  $p < 0.001$ ). The mothers were also more likely to be present in the unit than the fathers (90% vs. 77%, OR 3.0 [95%CI 2.3-4.0],  $p < 0.001$ ). Nurses gave 11 132 answers (response rate 55% of work shifts). They worked with parents in 79% (range 67%-90%) of their shifts. Their evaluation matched well with the parents' perceptions (5.6 [95%CI 5.6-5.7]). The country specific results are presented in **Tab. 1**.

## CONCLUSIONS

Both parents and nurses reported high level of parental participation and support in all countries.

# Neonatal Brain Injury and Neuroprotection

## ABS 64

### INCIDENCE OF FOCAL PRETERM BRAIN INJURY IN A DUTCH CENTER SPECIALIZED IN TREATMENT OF EXTREMELY PRETERM BIRTH

R. de Goederen<sup>1</sup>, R. de Jonge<sup>1</sup>, P. Govaert<sup>1</sup>, M. Feijen-Roon<sup>1</sup>, I. Reiss<sup>1</sup>, J. Dudink<sup>1,2,3</sup>

<sup>1</sup>Department of Neonatology, Erasmus MC-Sophia, Rotterdam, the Netherlands

**Table 1 (ABS 63).** Parents' and nurses' evaluations on parental participation and received support.

Participant/ Country	Mothers				Fathers				Nurses		
	Mothers (n)	Answers (n)	Present (%)	Level of participation and received support (Scale 1-7) Mean (95%CI)	Fathers (n)	Answers (n)	Present (%)	Level of participation and received support (Scale 1-7) Mean (95%CI)	Answers (n)	Worked with parents (%)	Level of participation and given support (Scale 1-7) Mean (95%CI)
Finland (1 NICU)	30	609	93	6.2 (5.9-6.4)	26	497	71	6.1 (5.8-6.4)	868	81	5.8 (5.7-5.9)
Sweden (3 NICUs)	72	1,370	83	6.0 (5.9-6.2)	66	1,202	79	6.0 (5.8-6.2)	2,627	88	5.9 (5.8-6.0)
Norway (3 NICUs)	67	1,239	94	6.0 (5.9-6.2)	54	1,071	77	5.9 (5.7-6.1)	3,363	80	5.9 (5.8-5.9)
Estonia (2 NICUs)	32	599	86	5.5 (5.2-5.7)	23	306	55	5.4 (5.0-5.7)	1,537	90	5.5 (5.4-5.6)
Spain (1 NICU)	32	645	98	5.8 (5.6-6.0)	20	34	89	5.4 (5.0-5.8)	2,737	67	5.6 (5.5-5.7)
Italy (1 NICU)	26	783	93	4.9 (4.7-5.2)	27	663	78	4.8 (4.5-5.1)	810	67	5.1 (4.9-5.2)
p-value			< 0.001	< 0.001			< 0.001	< 0.001		< 0.001	< 0.001



<sup>2</sup>Department of Pediatric Intensive Care, Erasmus MC-Sophia, Rotterdam, the Netherlands

<sup>3</sup>Department of Radiology, Erasmus MC-Sophia, Rotterdam, the Netherlands

## INTRODUCTION

Advances in neonatal care have reduced the high mortality rate in infants born very preterm worldwide. However, the number of ex-preterm infants with long-term developmental problems still remains high. In the past decades a shift from 'severe types of preterm brain injury' (such as cystic PVL) to less pronounced brain injury is seen.

Our aim was to report the incidence of focal preterm brain injury in our center, which is specialized in treatment of extremely preterms and treatment of maternal pre-eclampsia and has a focus on neonatal neuroimaging (ultrasound and MRI).

## PATIENTS AND METHODS

In the Erasmus MC-Sophia Children's Hospital, we prospectively recorded all infants with brain injury seen on routine neuroimaging admitted to the NICU in a neuro-database. Routine neuroimaging was performed according to a local protocol: cranial ultrasound scans were made on day 1, 2, 3, 7 and weekly onwards and MRI

scans were performed at 30 weeks gestation in all children born less than 29 weeks gestation. All images were reviewed by experienced researchers (JD, PG) and several maternal and neonatal clinical characteristics were recorded. Brain injury was classified as: IVH grade 1-2-3, PHVD, venous infarction, stroke and PVL.

## RESULTS

Between January 2009 and December 2014 a total of 1,728 infants born below 32 weeks gestation were admitted. Of these patients, 620 (35.9%) patients showed brain injury that fitted our classification, 345 (20.0%) patients had an IVH, 45 (2.6%) had PHVD and 54 (3.1%) had venous infarction. The number of patients with cerebellar hemorrhage, perforator stroke and sinus thrombosis were 36 (2.1%), 12 (0.7%) and 17 (1.0%), respectively. **Tab. 1** shows characteristics of the patients admitted to the NICU and incidence of brain injury within this population per year. There was a consistent number of patients admitted yearly and mean birth weight and mean gestational age remained consistent over the years. We saw slight increases of the incidence in cerebellar hemorrhage, sinus thrombosis, perforator strokes and IVH grade I and II.

**Table 1 (ABS 64).** Characteristics of infants born below 32 weeks of gestation admitted to the NICU and incidence of brain injury presented per year.

	2009	2010	2011	2012	2013	2014
Number of admissions	277	285	294	317	339	337
Re-admissions	11	16	19	26	20	29
First admissions	266	269	275	291	319	308
Male (%)	132 (49.6)	133 (49.4)	136 (49.5)	163 (56.0)	166 (52.0)	165 (53.6)
GA (weeks)	28.8 ± 2.3	28.7 ± 2.3	28.8 ± 2.3	28.8 ± 2.2	28.5 ± 2.3	28.9 ± 2.3
BW (grams)	1,156 ± 391	1,162 ± 391	1,198 ± 383	1,188 ± 368	1,169 ± 370	1,227 ± 432
BW < 1,000 grams (%)	103 (38.7)	103 (38.3)	87 (31.6)	107 (36.8)	113 (35.4)	98 (31.8)
Number of patients with brain injury (%)	62 (23.3)	68 (25.3)	97 (35.3)	119 (40.9)	97 (30.4)	106 (34.4)
IVH total	33	38	56	64	65	50
IVH grade I	14	12	17	24	27	23
IVH grade II	15	14	29	31	26	14
IVH grade III	4	12	10	9	12	13
Venous infarction	7	7	8	10	13	6
PHVD	6	7	7	8	4	7
PVL	1	1	3	1	6	7
Cystic PVL	0	1	2	0	1	3
Cerebellar hemorrhage	5	0	8	9	4	4
Sinus thrombosis	0	3	1	7	3	3
Perforator stroke	0	0	0	2	2	5
Other stroke	0	3	1	0	0	1

## CONCLUSIONS

In a highly selected population (a center specialized in pre-eclampsia and extremely preterm birth) we still see a high number of preterm infants with focal brain injury. Several factors will have contributed to this incidence, including the high standard of routine ultrasound scanning (Plaisier et al., 2014). The need for developing fetal and neonatal neuroprotective strategies and neurorehabilitative strategies remains high.

## ABS 65

### PREDICTION OF NEURODEVELOPMENTAL OUTCOME IN INFANTS WITH INTRAVENTRICULAR HEMORRHAGE USING A FUNCTIONAL MRI SCORE

K. Goeral<sup>1</sup>, G. Kasprian<sup>2</sup>, C. Leeb, R. Fuiko, A. Berger<sup>1</sup>, M. Olischar<sup>1</sup>, K. Klebermass-Schrehof<sup>1</sup>

<sup>1</sup>Department of Pediatrics and Adolescent Medicine – Division of Neonatology, Intensive Care and Pediatric Neurology, Medical University of Vienna, Vienna, Austria

<sup>2</sup>Department of Radiology – Division of Neuroradiology and Musculoskeletal Radiology, Medical University of Vienna, Vienna, Austria

## INTRODUCTION

Intraventricular hemorrhage (IVH) is a significant cause of morbidity and mortality in premature infants. There is a well known correlation between IVH grade and neurodevelopmental outcome. However, to our knowledge, there are only a few studies taking into account the site of the lesion. The aim of the present study was to create a tool which could serve as a prognostic indicator with regard to the severity of brain damage and long-term neurological outcome by assessing the functional areas affected by the bleeding.

## PATIENTS AND METHODS

64 infants with grade III IVH with and without parenchymal involvement who had MRI scans obtained during their clinical course and assessment of neurodevelopmental outcome were included into this analysis. MRI scans were analyzed by the computation of a composite grey matter (GMS), composite white matter (WMS) and a combined MRI (cS) score (composed of GMS, WMS and additional abnormalities) which included the functional areas described in **Tab. 1**. This standardised scoring system was developed in this study and consists of four-point scales for each area studied (0-3, increasing score with

**Table 1 (ABS 65).** MRI scans scores.

Grey Matter Score, GMS	
Gyrus precentralis	0-3
Gyrus postcentralis	0-3
Hippocampus	0-3
Basal ganglia	0-3
<b>Maximum points</b>	<b>12</b>
White Matter Score, WMS	
Pyramidal tract	0-3
Corpus callosum	0-3
Radiatio optica	0-3
Crossroad	0-3
<b>Maximum points</b>	<b>12</b>
Additional points	
Periventricular leukomalacia	0-3
Hydrocephalus	0-3
Cerebellum	0-3
<b>Maximum points</b>	<b>9</b>
Combined Scores, cS	
<b>Maximum points</b>	<b>33</b>

severity of the abnormality). Neurodevelopmental outcome was evaluated at 1 and 2 years corrected age via Bayley Scales of Infant development.

## RESULTS

There is a highly significant correlation between MRI scores and neurodevelopmental outcome: neonates with normal psychomotor developmental index (PDI) or mild disability show significantly lower GMS, WMS and cS compared to infants with moderate or severe psychomotor disability at 1 year and 2 year corrected age. The same is true for mental developmental index (MDI) at 1 year corrected age for all three scores. Correlations between MRI score and MDI/PDI at 1 year corrected are shown in **Fig. 1**.

## CONCLUSIONS

There is currently not much evidence with regard to the relevance of topography of injury when trying to predict long-term outcome in preterm infants with IVH. The proposed score might fill this gap and serve as a prognostic tool with regard to the severity of brain damage and long-term neurological outcome. It might therefore provide the clinician with invaluable information to improve individually tailored counselling in preterm infants with IVH.

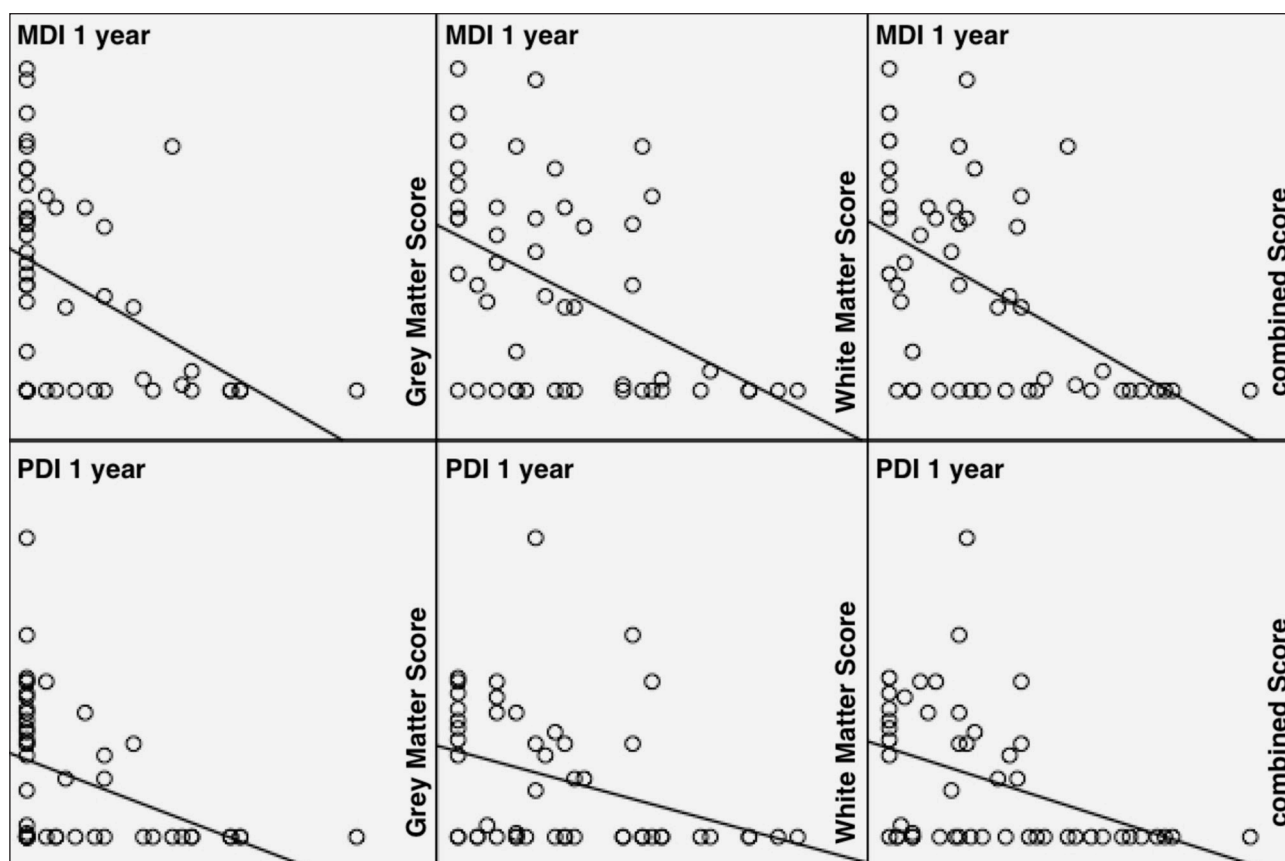


Figure 1 (ABS 65). Correlations between MRI score and MDI/PDI at 1 year corrected age.

## ABS 66

### SYSTEMIC HEMOLYSIS AND HYPEROXEMIA IN A RAT PUP MODEL; A NOVEL WAY OF MODELING EFFECTS OF CARDIOPULMONARY BYPASS

Å. Jungner, S. Vallius, M. Gram, D. Ley

Department of Pediatrics, Division of Clinical Sciences, Lund University, Lund, Sweden

#### INTRODUCTION

Rapid development in cardiac surgical care has enabled survival of an increasing number of infants with complex congenital heart defects. Unfortunately this is accompanied by a high prevalence of neurological and cognitive impairment diagnosed during childhood, with white matter damage as the dominant intracranial lesion detectable both pre- and postoperatively. In a postnatal day 6 (PN6) rat pup model we aim to evaluate two potentially damaging mechanisms – systemic hemolysis and hyperoxemia.

#### PATIENTS AND METHODS

Wistar wild-type litter-mixed PN6 rat pups were injected intraperitoneally with a weight-based dose of human cell-free hemoglobin. Animals were terminated at 0, 5, 1, 2, 3, 4, 5, 8, 12, 18 and 24 hours respectively with blood sampling and measurements of plasma free hemoglobin.

After confirming reproducible uptake litter-mixed animals were injected with weight-based dose of human cell-free hemoglobin or corresponding volume of vehicle only and subjected to 24 hours of normoxia ( $\text{FiO}_2$  0.21) or hyperoxia ( $\text{FiO}_2$  0.8). Animals were terminated at 3, 12 and 24 hours with blood sampling and harvesting of liver tissue on dry ice. mRNA and proteins were extracted from the livers with quantifications of markers of oxidative stress, DNA damage and repair, and systemic toxicity.

#### RESULTS

Maximal plasma-concentrations of cell-free hemoglobin were obtained 3 hours after injection reaching about 3 mg/ml. Elimination was biphasic with no measureable amounts of human cell-free hemoglobin detected at 24 hours.

No differences in uptake or oxidation of cell-free hemoglobin were detected between hyperoxic or normoxic groups. Gene array analysis on liver parenchyma showed enhanced expression of oxidative stress (Gstp1, Hmox1 and Nqo1) and DNA damage and repair (Hsp90, Hsp5, Nbn, Ripk3) genes in groups subjected to systemic hemolysis and hyperoxemia compared to groups subjected to vehicle and normoxia at 12 hours.

Measurements of baseline scavenger levels confirmed low levels of haptoglobin. After exposure to cell-free hemoglobin, haptoglobin levels were rising. Animals nested in hyperoxic environment displayed higher levels of pO<sub>2</sub> compared to animals nested in room air.

#### CONCLUSIONS

This novel PN6 rat pup model is a promising tool to evaluate consequences of systemic hemolysis and hyperoxemia in a newborn child. The insults are clinically relevant, making the model suitable for studies on intracranial and systemic effects following surgery on cardiopulmonary bypass.

#### ABS 67

#### BRAIN INJURY AND NEUROLOGICAL DEFICITS IN DIFFERENT MODELS OF HYPOXIA-ISCHEMIA IN NEONATAL MICE

B.S. Reinboth<sup>1</sup>, C. Köster<sup>2</sup>, K. Strasser<sup>3</sup>, I. Bendix<sup>4</sup>, U. Felderhoff-Müser<sup>5</sup>, J. Herz<sup>6</sup>

*Department of Pediatrics I, Neonatology, University Hospital Essen, Essen, Germany*

#### INTRODUCTION

Perinatal asphyxia is the main reason for hypoxic-ischemic encephalopathy (HIE), which is a significant cause of neonatal mortality and often leads to long lasting neurological deficits in newborn children. The most commonly used pre-clinical model for HIE is the Vannucci-Rice model, initially developed in rats and subsequently adapted to mice. However, results of pre-clinical studies testing clinically recommended therapies such as hypothermia demonstrate inconsistent effects which might be due to varying degrees of brain injury. Therefore, we tested different models of HIE in neonatal mice through variation of the hypoxic stimulus and ambient temperature.

#### PATIENTS AND METHODS

Hypoxia-ischemia (HI) was induced in postnatal day 9 C57BL/6 mice through ligation of the right common carotid artery followed by one hour hypoxia after one hour recovery with their dams. Hypoxia

was performed in an oxygen chamber at 8% or 10% oxygen (O<sub>2</sub>). Constant temperature was maintained through a warming blanket set at 37°C or 38°C. Body temperature was controlled with a rectal probe connected to a digital thermometer. 7 days post HI brains were analysed for regional tissue injury, neuronal loss, microtubuli associated protein 2 (MAP2) as well as myelin basic protein (MBP) expression using immunohistochemistry and Western Blot. Three weeks after HI motor coordination and spontaneous anxiety/exploration behaviour were evaluated using the RotaRod and the Elevated Plus Maze.

#### RESULTS

Based on the Vannucci-Rice rat model hypoxia was performed at 8% O<sub>2</sub> and 37°C resulting in mild brain injury and neuronal loss which was mainly confined to the hippocampus. MAP2 and MBP expression within complete hemispheres as well as functional outcome only partially differed compared to sham mice. Surprisingly, we detected a significant drop in body temperature during hypoxia. To maintain physiological body temperature during hypoxia the warming blanket temperature was set to 38°C leading to 50% mortality at 8% O<sub>2</sub>. Therefore, the hypoxic stimulus was reduced by increasing the O<sub>2</sub> concentration to 10%. Interestingly mortality rates strongly decreased to levels of the first model (37°C, 8% O<sub>2</sub>) but brain injury, neuronal loss and HI-induced functional deficits significantly increased. MAP2 as well as MBP expression were significantly reduced compared to sham operation and HI at 37°C, 8% O<sub>2</sub>.

#### CONCLUSIONS

Our data demonstrate that ambient temperature and oxygen concentration are the main predictors of mortality, brain injury and functional outcome. The observed differences in HI severity are apparently caused by an endogenous drop in body temperature during hypoxia. Therefore, maintenance of physiological temperature during the insult will be a prerequisite to test efficacy of therapeutic hypothermia and adjuvant therapies in neonatal mice.

#### ABS 68

#### COLD-INDUCIBLE RNA BINDING PROTEIN RBM3 PREVENTS ENDOPLASMIC RETICULUM (ER) STRESS-INDUCED APOPTOSIS

X. Zhu<sup>1</sup>, A. Zelmer<sup>1</sup>, J. Kapfhammer<sup>2</sup>, S. Wellmann<sup>1</sup>

*<sup>1</sup>Neonatology Department, University Children's Hospital of Basel (UKBB), Basel, Switzerland*

*<sup>2</sup>Biomedicine Department, University of Basel, Basel, Switzerland*



## INTRODUCTION

Moderate cerebral hypothermia is a potent therapeutic tool to ameliorate neural injury from various disorders, including hypoxic-ischemic encephalopathy (HIE) in newborn infants. The cold-inducible RNA-binding protein RBM3 belongs to a small group of proteins whose synthesis increases during hypothermia while global protein production is slowed down. RBM3 has been identified recently to be involved in the protection of neurons in hypoxic-ischemic and neurodegenerative disorders but little is known about the underlying molecular mechanisms.

## PATIENTS AND METHODS

Organotypic hippocampal slice cultures (OHSCs) from RBM3 knockout C57BL/6J mice and wild-type mice were subjected to various stressors, including oxygen glucose deprivation (OGD) and endoplasmic reticulum (ER) stress (e.g. triggered by thapsigargin), and cultured at 37°C or 32°C. Key components of ER stress response and apoptosis were assessed in OHSCs and various cell cultures by Western blot, real-time RT-PCR, propidium iodide staining, cell viability assay, and flow cytometry. To screen for RBM3 interaction partners, affinity purification coupled to mass spectrometry were performed; results were confirmed by co-immunoprecipitation and proximity ligation assay. Various specific siRNAs and expression plasmids were applied for functional tests.

## RESULTS

Moderate hypothermia promotes RBM3 expression and prevents brain from OGD-induced neuronal cell death. RBM3 associates with cell survival by inhibiting apoptosis, apparently involving Bcl-2 and cleaved PARP. Both moderate hypothermia and RBM3 inhibit the canonical ER stress pathway PERK-eIF2 $\alpha$ -CHOP by blocking PERK phosphorylation and consequently suppresses ER stress-induced apoptosis. In

RBM3 knock-out mice, PERK-eIF2 $\alpha$ -CHOP signalling was exacerbated compared with wild-type mice, particularly in hippocampus after ER stress induction. Among various candidate interaction partners of RBM3 identified in our screening, nuclear factor NF90 is proposed as a novel interactor of PERK and RBM3, and the presence of NF90 is required for RBM3-mediated regulation of PERK activity (**Fig. 1**).

## CONCLUSIONS

Our data suggest that under stressful conditions, hypothermia-induced RBM3 is protective in neural cells, and this protection is at least in part mediated by suppression of ER stress response. We propose a central role of RBM3 in preventing cell death by inhibiting the PERK-eIF2 $\alpha$ -CHOP signalling pathway through cooperation with NF90.

## ABS 69

### NEUROINFLAMMATION, A KEY FACTOR IN THE PATHOPHYSIOLOGY OF PRETERM INTRAVENTRICULAR HEMORRHAGE: THE ROLE OF MICROGLIA CELLS AND HAPTOGLOBIN AS A FEASIBLE THERAPY

A. Agyemang<sup>1</sup>, S. Vallius<sup>2</sup>, D. Ley<sup>3</sup>, M. Gram<sup>4</sup>

<sup>1</sup>Department of Pediatrics, Division of Clinical Sciences, Lund University, Lund, Sweden

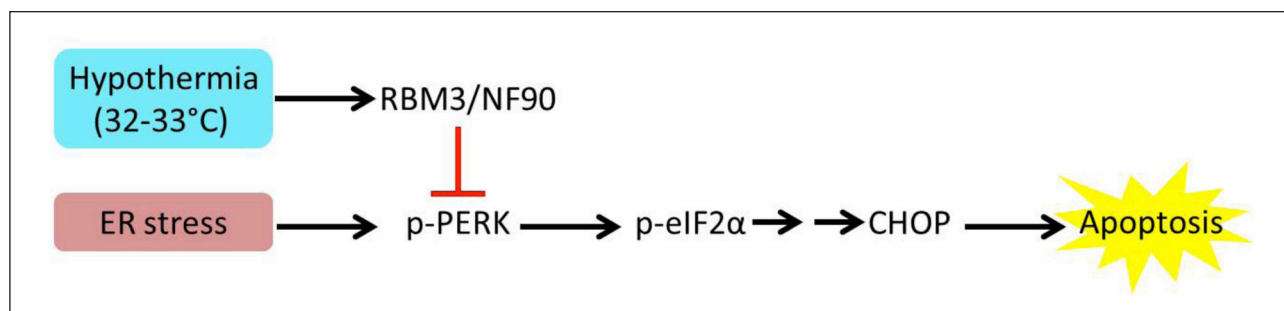
<sup>2</sup>Department of Pediatrics, Division of Clinical Sciences, Lund University, Lund, Sweden

<sup>3</sup>Department of Pediatrics, University Hospital of Lund, Lund, Sweden

<sup>4</sup>Department of Pediatrics, Division of Clinical Sciences, Lund University, Lund, Sweden

## INTRODUCTION

Preterm intraventricular hemorrhage (IVH) continues to be a major clinical problem and is the most common cause of neurological deficits



**Figure 1 (ABS 68).** Proposal of a central role of RBM3 in preventing cell apoptosis.

following preterm birth. To date there are no treatments available to diminish periventricular white matter brain damage following IVH. Inflammation has been shown to be a key event in development of periventricular damage. We have previously shown that cell-free hemoglobin and its metabolites is a key initiator of inflammation following IVH.

#### PATIENTS AND METHODS

The purpose of this study was to further characterize the molecular mechanisms of pro-inflammation following IVH by analyzing the response of microglia, the main resident immune cell of the brain, to cerebrospinal fluid (CSF) from infants with IVH. Further to assess possibilities to diminish the pro-inflammatory response of microglial cells. This was an *in vitro* study using an immortal human microglia cell line. The microglial cells were exposed to different concentrations (0-30%) of CSF from preterm infants with IVH. We further co-inoculated the cells with the hemoglobin scavenger, haptoglobin, in order to investigate a possibility protective effect. We evaluated the inflammatory response of the microglia cells by analyzing the expression of IL6, IL8 and MCP1 in the cell culture medium.

#### RESULTS

Analysis of the pro-inflammatory markers IL6, IL8 and MCP1 displayed a significant increase production following exposure to CSF from preterm infants with IVH. A stepwise increase in the cytokine concentration was seen following increasing CSF concentration, that is exposure to 10% CSF gave 167.0 pg/ml MCP1, 40.3 pg/ml IL6 and 5.8 pg/ml IL8 whilst exposure to 30% CSF gave 415.7 pg/ml MCP1, 70.9 pg/ml IL6 and 109.0 pg/ml IL8. Co-incubation with the hemoglobin scavenger haptoglobin, displayed a clear involvement of hemoglobin and/or hemoglobin metabolites since the addition of haptoglobin caused a clear decrease in the concentration of all pro-inflammatory markers.

#### CONCLUSIONS

Following IVH there is an activation of microglia, the resident immune cells of the brain. This study shows that there is an up-regulation of pro-inflammatory cytokines in microglia exposed to hemorrhagic CSF. This might contribute to the inflammatory cascade causing periventricular brain damage following IVH. Haptoglobin might be a feasible treatment option to diminish the effects of cell-free hemoglobin on pro-inflammation.

#### ABS 70

### FINGOLIMOD REDUCES NEONATAL WHITE MATTER DAMAGE AND LONG-TERM COGNITIVE DEFICITS

I. Bendix<sup>1</sup>, M. Serdar<sup>1</sup>, K. Kempe<sup>1</sup>, J. Herz<sup>1</sup>, K. Lumpe<sup>1</sup>, B.S. Reinboth<sup>1</sup>, S.V. Sizonenko<sup>2</sup>, X. Hou<sup>1,3</sup>, R. Herrmann<sup>1</sup>, M. Hadamitzky<sup>1</sup>, R. Heumann<sup>5</sup>, M. Sifringer<sup>6</sup>, Y.v.d. Looji<sup>2,7</sup>, U. Felderhoff-Müser<sup>1</sup>

<sup>1</sup>Department of Pediatrics I – Neonatology, University Hospital Essen, University Duisburg-Essen, Essen, Germany

<sup>2</sup>Department of Pediatrics, University of Geneva, Geneva, Switzerland

<sup>3</sup>Department of Pediatrics, Peking University First Hospital, Beijing, China

<sup>4</sup>Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University Duisburg-Essen, Essen, Germany

<sup>5</sup>Molecular Neurochemistry, Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Bochum, Germany

<sup>6</sup>Department of Anesthesiology and Intensive Care Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany

<sup>7</sup>Laboratory of Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

#### INTRODUCTION

Cerebral white matter injury is a leading cause of adverse neurodevelopmental outcome in prematurely born infants involving motor and cognitive function in later life. Despite increasing knowledge about the pathophysiology of perinatal brain injury, therapeutic options are limited. Experimental and clinical data from the adult demyelinating disease multiple sclerosis revealed beneficial effects of the sphingosine-1-phosphate receptor modulating substance fingolimod (FTY720). Herein, we evaluated the neuroprotective potential of FTY720 in a neonatal model of oxygen-toxicity leading to impaired neurocognitive outcome particularly emphasizing white matter development.

#### PATIENTS AND METHODS

The effect of FTY720 in hyperoxia-mediated white matter damage was assessed *in vitro* and *in vivo* focusing on (pre-)oligodendrocyte degeneration, maturation and differentiation by the use of immunohisto-, immunocytochemistry and flow cytometry. Oxidative stress parameters and local inflammatory responses were evaluated by high-performance liquid chromatography, real-time PCR, western blot and immunohistochemistry, respectively. Long-term motor and neurocognitive development was determined by behavioural testing. Structural alterations were investigated by diffusion weighted magnetic resonance imaging.

## RESULTS

Treatment with FTY720 reduces hyperoxia-mediated oxidative stress, local inflammation and oligodendrocyte degeneration. Oligodendrocyte maturation and differentiation is restored *in vitro* and *in vivo*. These cell-protective effects culminated in long-lasting neurocognitive and structural developmental improvements of adult rats after exposure to neonatal hyperoxia.

## CONCLUSIONS

Our data provide evidence that FTY720 might be a potential new therapeutic option for the treatment of neonatal brain injury through reduction of white matter damage.

## ABS 71

### NEURODEVELOPMENTAL OUTCOME AFTER EARLY HIGH DOSE RECOMBINANT HUMAN ERYTHROPOIETIN IN VERY PRETERM INFANTS: RESULTS OF A RANDOMIZED PLACEBO-CONTROLLED, DOUBLE-BLIND TRIAL

G. Natalucci<sup>1,2</sup>, B. Latal<sup>2</sup>, B. Koller<sup>1</sup>, C. Rüegger<sup>1</sup>, B. Sick<sup>3</sup>, L. Held<sup>3</sup>, H.U. Bucher<sup>1</sup>, J.C. Fauchère<sup>1</sup>; on behalf of the 'The Swiss EPO Neuroprotection Trial Group'

<sup>1</sup>Department of Neonatology, Zurich University Hospital, Zurich, Switzerland

<sup>2</sup>Child Development Centre, Zurich University Children's Hospital, Zurich, Switzerland

<sup>3</sup>Institute for Social and Preventive Medicine, Division of Biostatistics, University of Zurich, Zurich, Switzerland

## INTRODUCTION

Very preterm infants are at risk of developing encephalopathy of prematurity and consecutive long-term neurodevelopmental delay. Erythropoietin was shown to be neuroprotective in animal experimental and retrospective human clinical studies involving premature infants.

The aim of this study was to determine whether early high-dose recombinant human erythropoietin (rhEPO) treatment in preterm infants improves neurodevelopmental outcome at 24 months corrected age.

## PATIENTS AND METHODS

450 preterm infants between 26<sup>0/7</sup> and 31<sup>6/7</sup> gestational weeks were enrolled in a randomized, double-blind, placebo-controlled, multi-centre trial in Switzerland between 2005 and 2012. Participants were randomly assigned to receive rhEPO (3,000 IU/kg; n = 230) or placebo (NaCl 0.9%; n = 220) intravenously before 3

hours, at 12 to 18 hours, and at 36 to 42 hours after birth. The primary outcome of the trial was the cognitive development at 24 months corrected for prematurity, as defined by the mental development index (MDI) of the Bayley Scales of Infant Development, second edition (BSID-II). Secondary outcomes were motor development (PDI, BSID-II) and survival without mental retardation, cerebral palsy, severe auditory or visual impairment.

## RESULTS

At 24 months after term infants allocated to the high-dose rhEPO group had similar MDI, PDI, cerebral palsy, hearing or vision problems, and survival rates without impairment than infants allocated to the placebo group. The difference between the two groups did not reach statistical significance after adjustment in a per protocol analysis, e.g. after exclusion of those infants who did not get the allocated treatment or were treated with additional rhEPO for anaemia.

## CONCLUSIONS

Prophylactic early high dose rhEPO does not improve neurodevelopment of very preterm infants at 2 years. As EPO reduced brain injury assessed by MRI at term equivalent in a subgroup of study infants, the whole cohort will be followed up at 5 years.

## ABS 72

### THE HEME AND RADICAL SCAVENGER $\alpha$ 1-MICROGLOBULIN (A1M) CONFERS PROTECTION OF THE PERIVENTRICULAR WHITE MATTER FOLLOWING PRETERM INTRAVENTRICULAR HEMORRHAGE

S. Rutardottir<sup>1</sup>, S. Sveinsdottir<sup>2</sup>, S.R. Hansson<sup>1</sup>, B. Åkerström<sup>1</sup>, D. Ley<sup>1</sup>, M. Gram<sup>1</sup>

<sup>1</sup>Lund University, Lund, Sweden

<sup>2</sup>Lund University Hospital, Lund, Sweden

## INTRODUCTION

Cerebral intraventricular hemorrhage (IVH) is a major cause of severe neurodevelopmental impairment and mortality in very preterm infants. To date, no therapy is available that prevents or reduces the development of serious neurological disability. Following hemorrhage, extravasation of blood and release of extracellular hemoglobin (Hb) generates heme and free radicals that constitute pivotal events in the development of brain damage. Hb and its downstream metabolites are toxic and causal in inflammation, oxidative stress and cell death. We propose that the use of the heme and free radical scavenger  $\alpha$ 1-

microglobulin (A1M) might confer protection of the immature brain following preterm IVH.

#### PATIENTS AND METHODS

The objective of this study was to investigate the protective effect of A1M against white matter brain damage following IVH.

Using a preterm rabbit pup model of IVH, the structural and functional integrity, cellular, inflammatory and oxidative response of the periventricular white matter was evaluated at 24 and 72 hours following hemorrhage. Animals were injected with A1M and sham by ultrasound guided intraventricular injections and evaluated using mRNA and protein expression, immunohistochemistry and electron microscopy. In order to further investigate the protective effects of A1M, primary pre-oligodendrocytes, microglia and astrocytes were exposed to heme and free radicals with or without the addition of the A1M.

#### RESULTS

Results show that IVH in premature rabbit pups leads to an increased mRNA and protein expression of inflammation (TNF $\alpha$ , IL-1 $\beta$  and CCL2), and tissue injury mediators (MMP9) and cellular activation (TLR-4, COX-1, COX-2 and IL1R1) in periventricular brain tissue both 24 and 72 hours after bleeding has occurred. Intraventricular addition of A1M and congruent scavenging of heme and free radicals caused a significant reduction in inflammation, tissue injury and cellular response. *In vitro* studies showed that oligodendrocytes are highly sensitive to heme and radical exposure and that addition of A1M displayed a very protective effect.

#### CONCLUSIONS

Following IVH there is an increase of inflammation, tissue injury and cellular activation in the periventricular white matter. Administration of the heme- and radical scavenger A1M almost completely inhibited these effects. Exposure of oligodendrocytes to heme and radicals displayed a high sensitivity that was reduced with A1M. Our studies present an efficient means of decreasing the damage to the periventricular white matter following IVH.

#### ABS 73

#### VALIDATION OF CEREBRAL NIRS OXIMETRY IN NEWBORN PIGLETS

M. Rasmussen<sup>1,2</sup>, V. Eriksen<sup>1,2</sup>, S. Hyttel-Sorensen<sup>1</sup>, G. Greisen<sup>1</sup>

<sup>1</sup>Department of Neonatology, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark

<sup>2</sup>University of Copenhagen, Faculty of Health Sciences, Copenhagen, Denmark

#### INTRODUCTION

Near-infrared spectroscopy (NIRS) is increasingly being used to estimate cerebral tissue oxygenation in unstable neonatal infants.

Cerebral NIRS oximetry is often regarded as a trend monitor due to poor precision. In sick newborn infants a safe baseline oxygenation cannot be defined. The aim of this study was to validate both absolute values and changes in NIRS values against weighted cerebral saturation measured in arterial and venous blood in a piglet model.

#### PATIENTS AND METHODS

Continuous cerebral NIRS oximetry (NIRO-300, Hamatsu Phototonics) was conducted in 21 piglets at different levels of steady state hypotension. Hypotension was induced by gradually inflating a balloon catheter placed in vena cava, changing MAP in a stepwise manner.

The NIRS probe was placed above the left frontal and parietal cortex.

TOI is the oxygenated-hemoglobin to total-hemoglobin ratio in tissue a few centimeters beneath the sensor in an arteriovenous ratio of 1:2.

Absolute values and changes in TOI ( $\Delta$ TOI) were compared to weighted oxygen saturation ( $SwO_2 = 2/3 \cdot SvO_2 + 1/3 \cdot SaO_2$ ) of blood drawn from the superior sagittal sinus and aorta.

Linear regression, analysis of covariance (ANCOVA) and Bland-Altman plot was used to describe relation between TOI and  $SwO_2$  and between  $\Delta$ TOI and  $\Delta SwO_2$ .

#### RESULTS

Twenty-one piglets aging 4 to 66 hours were examined. PaCO<sub>2</sub> and arterial saturation were stable during measurements. Steady states of mean arterial blood pressure ranged from 14 to 82 mmHg.

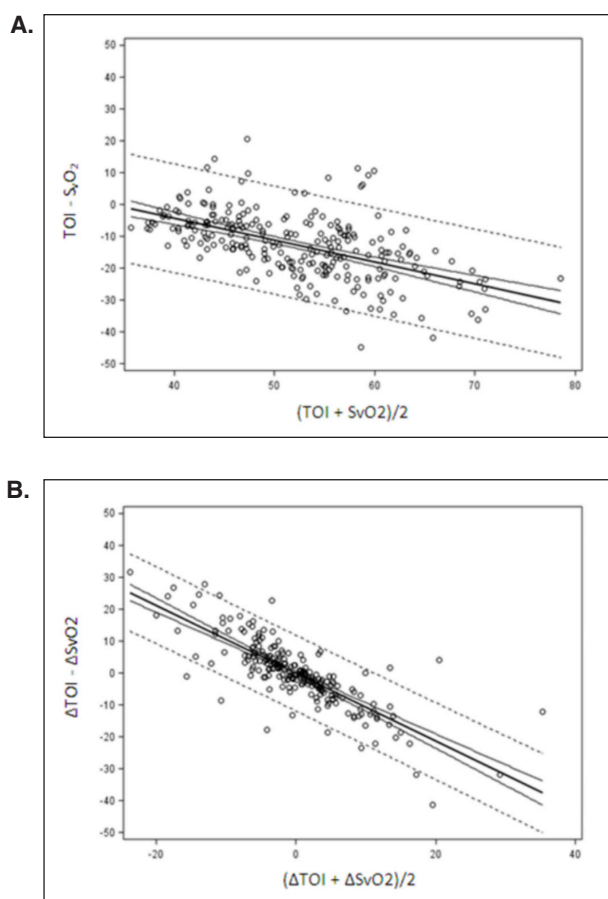
ANCOVA of TOI as a function of  $SwO_2$  revealed a regression coefficient of 0.27 (95% CI: 0.23-0.31,  $p < 0.001$ ).

Linear regression of  $\Delta$ TOI as a function of  $\Delta SwO_2$  revealed a regression coefficient of 0.23 (95% CI: 0.18-0.27,  $p < 0.001$ ).

Bland-Altman plots of TOI and  $SwO_2$  as well as  $\Delta$ TOI and  $\Delta SwO_2$  both showed a systematic relation between TOI and  $SwO_2$  with changing mean (**Fig. 1A** and **1B**).

95% Limit of agreement of the Bland-Altman regression lines were  $\pm 17.2\%$  and  $\pm 12.0\%$  for absolute values and changes in TOI and  $SwO_2$ , respectively (**Fig. 1A** and **1B**).





**Figure 1 (ABS 73).** Relation between TOI and  $SwO_2$ .

## CONCLUSIONS

The study show considerable uncertainties associated with interpreting NIRS values and trends as a change in cerebral oxygenation in newborn piglets. Difference between absolute and changes in values might be as big as  $\pm 17.2\%$  and  $\pm 12.0\%$ , respectively. Regression coefficients of 0.27 and 0.23 show that absolute TOI values and changes were less responsive than  $SwO_2$ , leaving both absolute and change coefficients far from the expected value of 1.

# Neurodevelopmental Outcome

## ABS 74

### BRAIN INJURY IN THE INTERNATIONAL MULTICENTRE RANDOMISED SafeBoosC PHASE II FEASIBILITY TRIAL: CRANIAL ULTRASOUND AND MAGNETIC RESONANCE IMAGING ASSESSMENTS

A.M. Plomgaard<sup>1</sup>, C. Hagmann<sup>2</sup>, T. Alderliesten<sup>3</sup>, T. Austin<sup>4</sup>, F. van Bel<sup>3,4</sup>, O. Claris<sup>5</sup>, E. Dempsey<sup>6</sup>, A. Franz<sup>7</sup>, M. Fumagalli<sup>8</sup>, C. Glud<sup>9</sup>, G. Greisen<sup>1</sup>, S. Hyttel-Soerensen<sup>1</sup>, P. Lemmers<sup>3</sup>, A. Pellicer<sup>10</sup>, G. Pichler<sup>11</sup>, M. Benders<sup>3</sup>

<sup>1</sup>Department of Neonatology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

<sup>2</sup>Department of Neonatology, University of Zurich, Zurich, Switzerland

<sup>3</sup>Universitair Medisch Centrum Utrecht, Wilhelmina Children's Hospital, Utrecht, the Netherlands

<sup>4</sup>Rosie Maternity Hospital Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

<sup>5</sup>Department of Neonatology, Hopital Femme Mere Enfants, Bron, France

<sup>6</sup>Department of Paediatrics and Child Health, University College Cork, Cork, Republic of Ireland

<sup>7</sup>Department of Neonatology, University of Tuebingen, Tübingen, Germany

<sup>8</sup>NICU, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

<sup>9</sup>Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

<sup>10</sup>Department of Neonatology, La Paz University Hospital, Madrid, Spain

<sup>11</sup>Department of Pediatrics, Medical University of Graz, Graz, Austria

## INTRODUCTION

Abnormal cerebral perfusion during the first days of life in preterm infants is associated with higher grades of intraventricular haemorrhages and lower 2-year developmental-score. In SafeBoosC II, we obtained a significant reduction of cerebral hypoxia by monitoring cerebral oxygenation in combination with a treatment guideline. Here we describe (i) difference in brain injury between groups, (ii) feasibility of serial cranial ultrasound (cUS) and MRI, (iii) local and central cUS-assessment.

Two-hundred-eight women in labor with singleton fetuses (< 32 weeks gestation) were randomized to either ICC (< 10 seconds) or DCC (30-45 seconds). The primary outcomes were (IVH), late onset sepsis (LOS) and motor outcomes at 18-22 months corrected age.

## PATIENTS AND METHODS

166 extremely preterm infants were included. cUS was scheduled for day 1, 4, 7, 14, 35 and at term-equivalent age (TEA). cUS was assessed locally (unblinded) and centrally (blinded). MRI at TEA was assessed centrally (blinded). Brain injury classification: no, mild/moderate, or severe.

## RESULTS

Severe brain injury did not differ between groups: cUS (experimental 10/80, control 18/77,  $p = 0.32$ ) and MRI (5/46 vs. 3/38,  $p = 0.72$ ). Kappa-values for local and central readers were moderate-to-good

for severe and poor-to-moderate for mild/moderate injuries. At TEA 72% of the infants were assessed by cUS and 64% by MRI.

#### CONCLUSIONS

No difference in brain injury between groups. Acquiring cUS and MRI data according the standard-operating-procedure could be improved. Whether monitoring cerebral oxygenation for the first 72 h of life prevents brain injury, needs to be evaluated in larger multicenter trials.

#### ABS 75

##### DELAYED CORD CLAMPING AT BIRTH IMPROVES MOTOR SCORES AT 18 TO 22 MONTHS CORRECTED AGE: A RANDOMIZED CONTROLLED TRIAL

J. Mercer<sup>1,2,3</sup>, D. Erickson-Owens<sup>1,2,3</sup>, B. Vohr<sup>2,3</sup>, R. Tucker<sup>2</sup>, W. Oh<sup>2,3</sup>, J. Padbury<sup>2,3</sup>

<sup>1</sup>University of Rhode Island, Kingston, RI, USA

<sup>2</sup>Alpert School of Medicine, Brown University, Providence, RI, USA

<sup>3</sup>Women and Infants Hospital of Rhode Island, Providence, RI, USA

#### INTRODUCTION

Preterm infants with delayed cord clamping (DCC) have shown less intraventricular hemorrhage (IVH) compared with immediate cord clamping (ICC). DCC may improve motor outcomes at 18-22 months.

#### PATIENTS AND METHODS

Two-hundred-eight women in labor with singleton fetuses (< 32 weeks gestation) were randomized to either ICC (< 10 seconds) or DCC (30-45 seconds). The primary outcomes were (IVH), late onset sepsis (LOS) and motor outcomes at 18-22 months corrected age.

#### RESULTS

Cord clamping time was  $6.6 \pm 6$  (ICC) vs.  $32 \pm 16$  seconds (DCC). Infants in the ICC and DCC groups weighed  $1,136 \pm 350$  and  $1,203 \pm 352$  grams and mean gestational age was  $28.4 \pm 2$  and  $28.3 \pm 2$  weeks respectively. There were no differences in rates of IVH or LOS between groups. At 18-22 months, DCC was protective against motor scores below 85 on the Bayley Scales of Infant Development-III (Bayley-III) (OR 0.32, 95% CI 0.10-0.90,  $p = 0.03$ ). Sensitivity analyses verified the robustness of the findings. There were more women with preeclampsia (PEC) in the ICC group (37% vs. 22%,  $p = 0.02$ ) and more women in the DCC group with premature rupture of membranes/

preterm labor (PROM/PTL) (54% vs. 75%,  $p = 0.002$ ). PEC halved the risk of IVH (OR 0.50, 95% CI 0.2-1.0) and PROM/PTL doubled the risk (OR 2.0, 95% CI 1.2-4.3).

#### CONCLUSIONS

Although DCC did not alter the incidence of IVH or LOS in preterm infants in this study, it improved motor function at 18-22 months corrected age.

#### ABS 76

##### VOICE OUTCOMES FOLLOWING VERY PRE-TERM BIRTH

S. Meldrum<sup>1,2</sup>, K. Simmer<sup>3,4</sup>, S. Vijayasekaran<sup>1,5,6</sup>, N. French<sup>3,4,7</sup>

<sup>1</sup>School of Paediatrics and Child Health, University of Western Australia, Perth, WA, Australia

<sup>2</sup>School of Psychology and Social Science, Edith Cowan University, Perth, WA, Australia

<sup>3</sup>Department of Neonatal Paediatrics, King Edward Memorial Hospital and Princess Margaret Hospital for Children, Subiaco, WA, Australia

<sup>4</sup>Centre for Neonatal Research and Education, University of Western Australia, Perth, WA, Australia

<sup>5</sup>Department of Otolaryngology and Head and Neck Surgery, Princess Margaret Hospital for Children, Subiaco, WA, Australia

<sup>6</sup>School of Surgery, University of Western Australia, Perth, WA, Australia <sup>7</sup>State Child Development Centre, Health Department of Western Australia, Perth, WA, Australia

#### INTRODUCTION

Dysphonia is a potential, long-term complication of extreme preterm birth, with neonatal intubation being a strongly influencing factor. Risk factors correlated with adverse voice outcomes include female gender, multiple intubations, extremely low birth weight, complicated intubation procedure, surgical ligation of patent ductus arteriosus and extreme prematurity. Very preterm children may also be exposed to factors associated with disturbances in voice quality.

This study sought to prospectively evaluate the incidence of dysphonia in very preterm children, at school age, with reference to a term-born group of children with no risk for voice problems from the same community.

#### PATIENTS AND METHODS

Preterm participants were recruited from electronic records at King Edward Memorial Hospital. Participants were randomly selected, by medical record number, following stratification into groups based on gestational age and number of intubations. Medical and demographic information, including

intubation history, was abstracted from patient charts. Term-born participants were recruited from a community sample.

Clinical voice assessment consisted of:

- perceptual evaluation with the Consensus Auditory-Perceptual Analysis of Voice (CAPE-V) rating scale;
- computerised analysis of the voice signal with the Acoustic Voice Quality Index (AVQI);
- caregiver-proxy quality of life evaluation with the 27-item Pediatric Voice Handicap Index (pVHI).

## RESULTS

Out of 391 very preterm children approached, 178 completed voice assessments. The incidence of dysphonia in this cohort was 62.6%. The incidence of dysphonia in the term-born reference group was 23.09%. The incidence of dysphonia decreased with increasing gestational age, but was higher in the preterm group at each gestational age than the reference group.

Univariable analysis of factors associated with the presence of dysphonia in the very preterm group demonstrated that total number of intubations, maximum tube size and tube size to bodyweight ratio were significantly associated with voice difficulties. However, only female gender, gestational age and duration of intubation was significant in the multivariable model. The incidence of dysphonia in children who were never intubated was 42.5%, and included 7.5% with moderate dysphonia (c.f. 1.5% in the term-born group).

## CONCLUSIONS

Preterm children showed much more significant voice abnormalities at school age than their term-born peers. Voice problems limiting social and academic success may be experienced by some very preterm children who are otherwise well.

Dysphonia is clearly associated with very preterm birth. We do not know why females are more susceptible or the mechanisms underlying voice problems in children who were never intubated and further research is needed.

## ABS 77

### TIMING OF AMPLITUDE-INTEGRATED ELECTROENCEPHALOGRAPHY RECORDING FOR PREDICTION OF OUTCOME IN PREMATURE INFANTS

E. Ralser, V. Neubauer, U. Pupp-Peglow, U. Kiechl-Kohlendorfer, E. Griesmaier

*Department of Paediatrics II, Neonatology, Medical University, Innsbruck, Austria*

## INTRODUCTION

Despite an increasing survival rate of very preterm infants neurologic morbidity is still a major concern. The prediction of their outcome to counsel parents and to make decisions for further therapies needs reliable diagnostic tools. Amplitude-integrated electroencephalography (aEEG) has already proved to predict outcome of asphyxiated term infants. Other studies showed its value to predict neurodevelopmental outcome in preterm infants < 32 weeks gestational age suffering from intracranial bleeding. The aim of this study was to evaluate the well-established Burdjalov Score as an objective parameter to predict neurodevelopmental outcome at the age of 12 months in very preterm infants.

## PATIENTS AND METHODS

Infants born < 32 completed weeks of gestation between October 2007 and December 2013 were prospectively enrolled. All infants were admitted to the University Hospital Innsbruck the only neonatal intensive care unit of Tyrol and all parents were resident within the study area. Preterm babies with major congenital anomalies and congenital infections were excluded. The Bayley Scale of Infants Development II was used to quantify psychomotor (PDI) and mental (MDI) developmental indices at the corrected age of 12 months. aEEG recordings were performed within the first 72 hour of life and then weekly till the age of 4 weeks. For comparing the Burdjalov Score and neurologic outcome parametric and non-parametric tests were used as appropriate. ROC curves were created to predict outcome.

## RESULTS

232 infants with neurodevelopmental follow up at 12 months corrected age were investigated. This study population was divided into normal outcome: MDI and PDI > 85 (n = 154), moderate delay: PDI or MDI 71-84 (n = 53) and severe delay: PDI or MDI < 70 (n = 25). In all three groups the Burdjalov score increased over the first 4 weeks of life. Scores were lower in infants with moderate delay than in infants with normal outcome and even lower in infants with severe delay. While comparing scores of all three outcome groups the most significant differences were found within the first 72 hours of life emphasising 18-24 hours and 30-36 hours of life (p-value 0.003 vs. 0.001). Similar pattern were found in all subgroup analysis, as outcome of PDI, MDI or single score evaluations. Further, the

Burdjalov Score presented the highest AUC at 18-24 hours of life (AUC 0.776).

## CONCLUSIONS

Our study confirmed the predictive value of the aEEG and acknowledged a maturation process in all outcome groups over the first 4 weeks of life. The best correlation between the Burdjalov score and outcome was found on the first day of life. We emphasize that aEEG monitoring needs to be done early in life to give reliable information about neurodevelopmental outcome in preterm infants.

## ABS 78

### BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT (EDITION 3) IN A LOW RISK HEALTHY POPULATION

C. Ahearne<sup>1</sup>, G. Hannon<sup>1</sup>, M. Kiely<sup>1</sup>, L. Kenny<sup>1</sup>, J.O'B. Hourihane<sup>2</sup>, D. Murray<sup>1,2</sup>

<sup>1</sup>Irish Centre for Fetal and Neonatal Translational Research, UCC, Cork, Ireland

<sup>2</sup>Department of Paediatrics and Child Health, Cork University Hospital, Cork, Ireland

## INTRODUCTION

The Bayley Scales of Infant and Toddler Development (Edition 3) [BSID-III] is a widely used developmental assessment tool for the early detection of disability in high-risk groups. However it was standardised on an American paediatric population, which included 10% from at-risk populations. Recently concerns regarding population differences have been raised. In addition the inclusion of high-risk populations may lead to the underestimation of developmental delay. The aim of this study was to determine the performance of a low-risk European population in the BSID-III compared with standardised normative values.

## PATIENTS AND METHODS

A representative sample of children were chosen from within the population based Cork BASELINE birth cohort study and invited to attend for neurodevelopmental assessment using BSID-III at 2 years. Only healthy, low risk singleton infants with no perinatal risk factors were included in analysis. Exclusion criteria were IUGR, prematurity, HIE and congenital anomalies or failure to complete any subtest. Analysis used t-tests to compare mean scores for each scale with standardised norms. Scaled scores were compared to a test value (SD) of 10 (3) and composite scores were compared to a test value of 100 (15).

## RESULTS

240 children were assessed using the BSID-III at a median (min-max) age of 27 months 5 days (24 months 13 days - 32 months 28 days). 198 children had completed all subscales of the BSID-III and 42 assessments were incomplete. Language composite scores (mean  $\pm$  SD) were significantly higher compared to standardised normative values,  $109 \pm 13$  v.  $100 \pm 15$ ,  $p < 0.001$ . This was based on raised receptive and expressive language scaled scores,  $11.2 \pm 2$  v.  $10 \pm 3$ ,  $p < 0.001$  and  $11.8 \pm 3$  v.  $10 \pm 3$ ,  $p < 0.001$  respectively. Fine motor scaled scores (mean  $\pm$  SD) also increased significantly,  $11.5 \pm 2$  v.  $10 \pm 3$ ,  $p < 0.001$  which had an effect on overall motor composite score,  $106 \pm 11$  v.  $100 \pm 15$ ,  $p < 0.001$  though gross motor scaled scores did not differentiate significantly,  $p = 0.151$ . Cognitive composite scores also did not show a significant difference from norms,  $p = 0.214$  (**Tab. 1**).

## CONCLUSIONS

This is the first known data on the performance of a European population of low-risk 2-year olds on the BSID-III. These results further highlight the existence of population variations. These results

**Table 1 (ABS 78).** Comparison of population scores to normative values.

Subtest score	N	Mean difference (95% CI)	P-value
Cognitive scaled score	234	-0.171 (-0.44, 0.10)	0.214
Receptive language scaled score	214	1.238 (0.95, 1.53)	< 0.001
Expressive language scaled score	209	1.766 (1.38, 2.15)	< 0.001
Fine motor scaled score	230	1.496 (1.19, 1.8)	< 0.001
Gross motor scaled score	216	0.250 (-0.09, 0.59)	0.151
Cognitive composite score	234	-0.855 (-2.21, 0.50)	0.214
Language composite score	208	9.192 (7.39, 11.00)	< 0.001
Motor composite score	212	5.807 (4.28, 7.33)	< 0.001



also suggest that we are at risk of underestimating developmental delay in language and fine motor skills if relying on U.S. standardised scores with whole population norms.

## ABS 79

### EARLY POSTNATAL HYDROCORTISONE IMPAIRS CEREBELLAR GROWTH AND DELAYS NEONATAL AROUSAL IN PRETERM PIGS

A. Brunse, P. Worsøe, M. Birck, P. Sangild

Section of Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark

#### INTRODUCTION

Preterm infants are frequently administered glucocorticoids (GC) to prevent bronchopulmonary dysplasia after prolonged periods of mechanical ventilation. Early treatment may benefit initial respiratory function but especially high-dose, long-acting GC treatment is suspected to induce adverse neurological and intestinal effects (e.g. cerebral

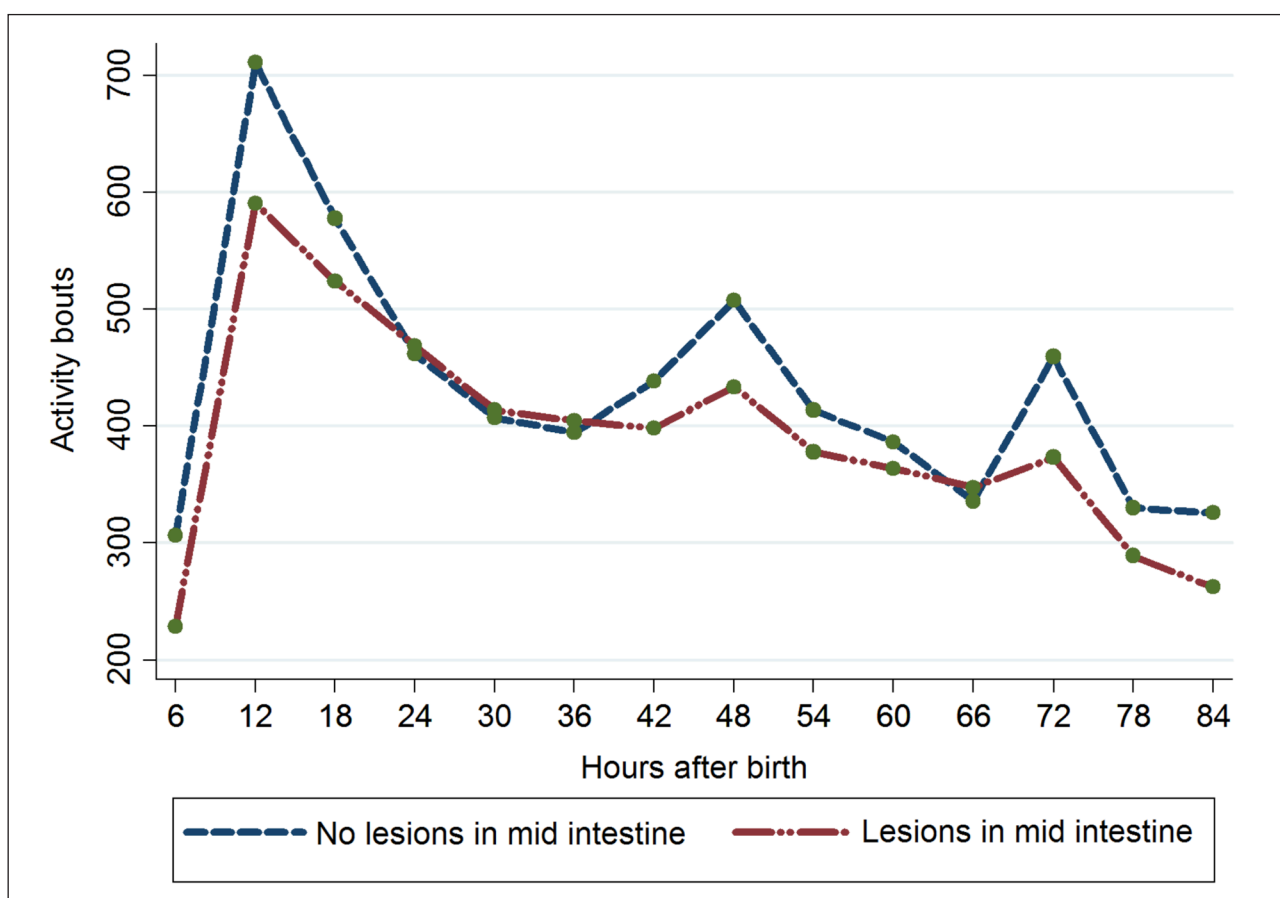
palsy and intestinal perforation). We hypothesized that short-acting postnatal hydrocortisone (HC) treatment is not associated with such negative effects, using preterm pigs as a model for preterm infants.

#### PATIENTS AND METHODS

We delivered thirty-five piglets from two sows by cesarean section at 90% gestation. Piglets were randomized to receive clinically relevant, diminishing doses of HC (6 → 2 mg/kg/d, n = 18) or equivalent saline doses (CTRL, n = 17) for four days beginning immediately after birth. We quantified home-cage activity (HCA) by continuous video recordings and registered eye-lid opening and first stand. We euthanized the piglets at postnatal day 5, macroscopically assessed gastrointestinal pathology, and collected the brains for regional weights (cerebrum, cerebellum and stem) and hydration level.

#### RESULTS

HC cerebella were smaller than CTRL ( $2.60 \pm 0.05$  vs.  $2.74 \pm 0.04$  g,  $p < 0.05$ ) and cerebellar fraction of whole brain was reduced ( $p < 0.01$ ). Body weight, brain weight and brain water content did not differ.



**Figure 1 (ABS 79).** Home-cage activity (HCA) during postnatal period in preterm with and without lesions in mid intestine.

HC piglets were slower to acquire the ability to stand after birth ( $45 \pm 6$  vs.  $28 \pm 2$  h,  $p < 0.05$ ), with no difference in eye lid opening. HC piglets had a higher frequency of hemorrhagic lesions in the mid intestine ( $p < 0.01$ ) with no differences in other regions (stomach, proximal and distal intestine, colon). Piglets with lesioned mid intestines had a significantly lower HCA level ( $p < 0.05$ , **Fig. 1**).

#### CONCLUSIONS

After just four days of HC treatment, we demonstrated impaired growth of the rapidly developing cerebellum accompanied by delayed neonatal arousal. Furthermore, we identified a distinct pattern of small bowel hemorrhagic lesions in the HC pigs. Low HCA level was predictive of later occurring lesions as early as postnatal day 1. We conclude that early postnatal HC treatment is associated with both brain and gut adverse effects.

#### ABS 80

#### ALTERED SLEEP SPINDLE ACTIVITY MAY REFLECT IMPAIRED THALAMOCORTICAL CONNECTIVITY IN VERY PRETERM CHILDREN AND ADOLESCENTS

F. Wehrle<sup>1,2</sup>, B. Latal<sup>2</sup>, R. O’Gorman<sup>3</sup>, C. Hagmann<sup>1</sup>, R. Huber<sup>2,4</sup>

<sup>1</sup>Department of Neonatology, University Hospital Zurich, Zurich, Switzerland

<sup>2</sup>Child Development Center, University Children’s Hospital Zurich, Zurich, Switzerland

<sup>3</sup>Center for MR Research, University Children’s Hospital Zurich, Zurich, Switzerland

<sup>4</sup>University Clinic for Child and Adolescent Psychiatry, Zurich, Switzerland

#### INTRODUCTION

The majority of very preterm infants survive without any severe neurodevelopmental impairments, however, up to 50% of all very preterm children suffer from subtle cognitive deficits and learning problems. Very preterm birth is frequently associated with altered development of the thalamocortical system. Sleep spindles, thalamocortically generated phasic oscillations between 12 and 15 Hz during non-rapid eye movement (NREM) sleep, reflect the integrity of the thalamocortical system and are associated with general cognitive abilities and learning. The current study aimed to investigate potential alterations in the topographical distribution of sleep spindle activity in very preterm children.

#### PATIENTS AND METHODS

Thirty-eight very preterm participants ( $29.5 \pm 2.1$  [M  $\pm$  SD] weeks of gestation) without any severe brain injuries and forty-three healthy term-born peers were assessed at a mean age of  $13.1 \pm 1.9$  years. All-night high-density sleep EEG (128 electrodes) was recorded in all participants. Power maps were calculated based on the average spindle activity of the first hour of NREM sleep.

#### RESULTS

Sleep efficiency was high in both groups (approximately 90%). The duration and architecture of sleep were not significantly different between the groups ( $p > .40$ ). In a widespread temporal/parietal cluster of 23 electrodes, very preterm participants exhibited significantly less spindle activity than their term-born peers (decrease of  $10.9 \pm 2.4\%$ ,  $p < .001$ ). Also, in a smaller frontal cluster of five electrodes, very preterm participants exhibited significantly more spindle activity (increase of  $14.3 \pm 0.7\%$ ,  $p = .02$ ). Lower spindle activity was associated with lower IQ in temporo-parietal brain regions, both in very preterm and term-born participants ( $p < .05$ ).

#### CONCLUSIONS

Very preterm children show an altered sleep spindle activity topography compared to term-born peers. This may indicate alterations in thalamocortical connectivity and impairments in cognitive abilities and learning. The reduction in spindle activity was observed over brain areas associated with cognitive domains (e.g., calculation) which are often impaired in very preterm children. The local increase over frontal brain areas may reflect more intense use of this area in very preterm children.

#### ABS 81

#### REGIONAL CEREBRAL OXYGENATION AS MEASURED BY NEAR-INFRARED SPECTROSCOPY (NIRS) IS RELATED TO NEURODEVELOPMENTAL OUTCOME AT 2 YEARS OF AGE

T. Alderliesten, I.C. van Haastert, W. Baerts, N.E. van der Aa, L.S. de Vries, C. Koopman, F. van Bel, P.M.A. Lemmers

Department of Neonatology, University Medical Center Utrecht/Wilhelmina Children’s Hospital Utrecht, Utrecht, the Netherlands

#### INTRODUCTION

Neonates admitted to the neonatal intensive care (NICU) are closely monitored to ensure adequate

oxygen and blood supply to vital organs, most importantly the brain. Although, the arterial blood pressure is one of the most commonly monitored parameters, accumulating evidence suggests that monitoring blood pressure alone might not be sufficient to ensure adequate brain perfusion. Regional cerebral oxygen saturation (rScO<sub>2</sub>) as measured by NIRS might be a better parameter to monitor the oxygen and blood supply to the brain. The aim of the current study was to monitor the rScO<sub>2</sub> in the first days after birth and determine whether it was related to neurodevelopmental outcome at 2 years of age

#### PATIENTS AND METHODS

As part of a prospective observational cohort study, rScO<sub>2</sub> was monitored simultaneously with other vital parameters in all infants with a gestational age (GA) < 32 weeks who were admitted to the NICU of the UMC Utrecht in the first 72 hours after birth. Neurodevelopmental outcome was assessed according to the standard follow-up protocol in our hospital: Bayley Scales of infant and toddler development 2<sup>nd</sup> or 3<sup>rd</sup> edition (BSITD-II/III-NL), depending on the year of birth, for infants < 30 weeks GA, and Griffiths Mental Development Scales (GMDS) for those with a GA 30-32 weeks.

#### RESULTS

Between 2005-2013, monitoring was intended in 1,059 infants. In 999 infants rScO<sub>2</sub> data were successfully obtained, 539 boys, 460 girls, mean GA 28.7 weeks (SD 2.0), mean birth weight of 1,150 g (SD 330). Seventy-five infants died, GMDS was performed in 699, BSITD II-NL in 62, and BSITD III-NL assessment in 444 infants. Mean BSITD III-NL cognitive score was 102 ± 14 corrected and 93.2 ± 12 not corrected for preterm birth. Mean GMDS cognitive score was 103 ± 10.

The rScO<sub>2</sub> on day 3 had a quadratic association with BSITD III-NL scores ( $p < 0.001$ ), suggesting that both a rScO<sub>2</sub> in the lower or in the higher regions were associated with lower BSITD-III-NL scores. The same applied to the association between rScO<sub>2</sub>'s on days 1, 2, and 3 (all  $p < 0.001$ ) and GMDS performance. Furthermore, infants who died during the neonatal period had lower rScO<sub>2</sub>'s on days 1, 2, and 3 (all  $p < 0.001$ ).

#### CONCLUSIONS

The strong association of rScO<sub>2</sub> with neurodevelopmental outcome confirms the place of NIRS on the NICU. The identification of clear thresholds associated with adverse outcome should be identified.

#### ABS 82

#### A 3D MAP OF ISCHEMIC STROKE FREQUENCY IN THE NEONATAL BRAIN

C. Stephan-Otto<sup>1</sup>, G. Arca-Diaz<sup>2</sup>, A. Garcia-Alix<sup>2</sup>

<sup>1</sup>Unitat de Recerca, Parc Sanitari Sant Joan de Deu, FSJD, University of Barcelona. CIBERSAM, Barcelona, Spain

<sup>2</sup>Neonatology, Agrupacion Sanitaria Sant Joan de Deu, Hospital Clinic, BCNatal, University of Barcelona, Barcelona, Spain

#### INTRODUCTION

Neonatal Arterial Ischemic Stroke (AIS) presents wide variability both in lesion extent and patient outcome. Magnetic Resonance Imaging is useful in prognosis of motor impairment but higher cognitive deficits are not well predicted. There are scarce studies about the distribution of AIS, the first step to establish an accurate relationship between injury and outcome. The proposed approach could shed light on the lesion to symptom relationship in neonatal AIS.

#### PATIENTS AND METHODS

Lesion volumes of AIS were calculated semi-automatically for 25 neonatal brains, using the multimodal segmentation function in ITK-SNAP software. Simultaneous inspection of T1, T2 and DWI acquisitions allowed for a precise definition of lesion extent. Individual lesion images were warped with ANTs software into a standardized neonatal brain by elastically registering individual T1 images to a T1 template. The superposition of the resulting standardized lesions was then visualized with MRICroN software.

#### RESULTS

A heat map depicting the spatial distribution of lesions for the whole brain was created. The angular and supramarginal gyri were among the most frequently affected regions. Although the distribution of lesions was essentially bilateral, we observe a slight tendency towards the left hemisphere (**Fig. 1**).

#### CONCLUSIONS

Our findings indicate regions with increased susceptibility to neonatal AIS and may explain the prevalence of certain functional deficits as speech, behavioral and other cognitive disorders in this pathology. Follow-up studies based on lesion-symptom mapping are necessary for accurate outcome prediction and may open new targets for early and focalized rehabilitation therapy.

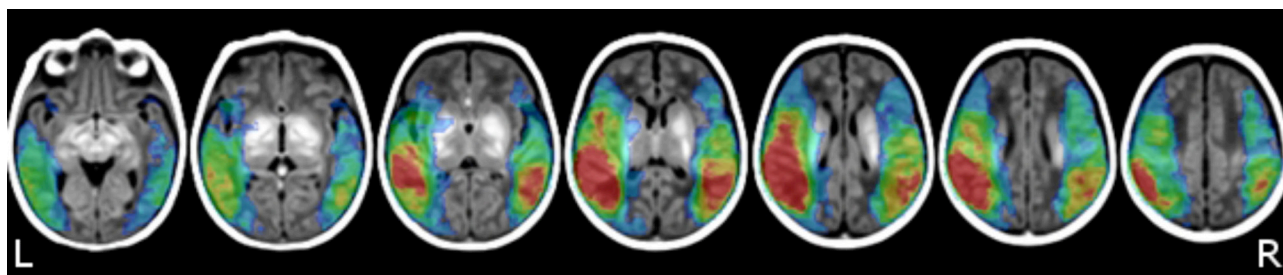


Figure 1 (ABS 82). Spatial distribution of lesions in ischemic stroke.

## Placenta and Prenatal Factors

### ABS 83

#### CONGENITAL HEART DISEASE AND INDICES OF FETAL CEREBRAL GROWTH IN A NATIONWIDE COHORT OF 931,174 LIVEBORN INFANTS

N.B. Matthiesen<sup>1</sup>, T.B. Henriksen<sup>1</sup>, J.W. Gaynor<sup>2</sup>, P. Agergaard<sup>1</sup>, C.C. Bach<sup>1</sup>, V. Hjortdal<sup>3</sup>, J.R. Ostergaard<sup>1</sup>

<sup>1</sup>Department of Pediatrics, Aarhus University Hospital, Aarhus University, Aarhus, Denmark

<sup>2</sup>Division of Cardiothoracic Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

<sup>3</sup>Department of Cardiothoracic Surgery, Aarhus University Hospital, Aarhus University, Aarhus, Denmark

#### INTRODUCTION

Congenital heart disease (CHD) is the most common major birth defect worldwide. Neurodevelopmental disorders are present in up to 50% of children with CHD and are considered to be the most distressful long term comorbidity. Fetal cerebral growth, especially head circumference at birth (HC), has consistently been associated with neurodevelopment from the neonatal period through school age. However, it remains unsettled which subtypes of CHD are associated with smaller HC at birth, and whether head size is small compared to overall size of the infant. We investigated the association between subtypes of CHD and size at birth in a large nationwide cohort.

#### PATIENTS AND METHODS

We conducted a nationwide cohort study including all Danish livebirths 1997-2012. Multiple pregnancies were excluded. CHD, HC, birth

weight (BW), gestational age at birth and potential confounders were identified in national registries. The diagnostic validity and the registration of genetic anomalies were validated in detail in 30% of infants with CHD. The associations between CHD, HC and BW z-scores for gestational age were analyzed by multivariable linear regression adjusted for potential confounders including: infant sex, parental origin, maternal age, bmi, hypertension, diabetes and smoking as well as the presence of infant extracardiac malformations, congenital syndromes and year of birth. To account for unmeasured genetic and socioeconomic factors we also conducted a sibling study.

#### RESULTS

931,174 livebirths were included. CHD was present in 7,799 infants. Overall, CHD was associated with a lower HC z-score -0.07 (95% CI -0.09;-0.05) and a lower BW z-score, -0.15 (95% CI -0.17;-0.12). Several subtypes were associated with a reduction of HC z-score: hypoplastic left heart syndrome -0.37 (95% CI -0.55;-0.20), other single ventricle defects -0.30 (95% CI -0.47;-0.13), transposition of the great arteries -0.32 (95% CI -0.46;-0.19), tetralogy of Fallot -0.35 (95% CI -0.49;-0.21), common arterial trunk -0.44 (95% CI -0.76;-0.12), double outlet right ventricle -0.37 (95% CI -0.69;-0.05), anomalous pulmonary venous return -0.43 (95% CI -0.76;-0.11) and major ventricular septal defects -0.26 (95% CI -0.36;-0.15). Only transposition of the great arteries was associated with a smaller HC compared to BW, z-score difference -0.27 (95% CI -0.40;-0.14). Results of the sibling study were consistent with these results.

#### CONCLUSIONS

This is the first study to investigate the association between all major subtypes of CHD and measures of fetal cerebral growth. We identified several subtypes of CHD not previously associated with decreased cerebral growth, including major ventricular septal defects. Impaired fetal cerebral growth was only an isolated cerebral phenomenon in infants with



transposition of the great arteries, consistent with models of preferential cerebral hypoxia.

## ABS 84

### CONGENITAL HEART DISEASE, PLACENTAL ANOMALIES AND INDICES OF FETAL GROWTH IN A NATIONWIDE COHORT OF 931,174 LIVE-BORN INFANTS

N.B. Matthiesen<sup>1</sup>, T.B. Henriksen<sup>1</sup>, J.W. Gaynor<sup>2</sup>, P. Agergaard<sup>1</sup>, C.C. Bach<sup>1</sup>, V. Hjortdal<sup>3</sup>, J.R. Ostergaard<sup>1</sup>

<sup>1</sup>Department of Pediatrics, Aarhus University Hospital, Aarhus University, Aarhus, Denmark

<sup>2</sup>Division of Cardiothoracic Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

<sup>3</sup>Department of Cardiothoracic Surgery, Aarhus University Hospital, Aarhus University, Aarhus, Denmark

## INTRODUCTION

Placental anomalies have recently been associated with fetal congenital heart disease (CHD), growth in fetuses with CHD, as well as neurodevelopmental disorders in children with CHD. However, earlier studies have been small, the associations with different subgroups of CHD have not been studied, and important confounders have not been accounted for. We aimed to investigate the association between all major subtypes of CHD and placental weight (PW) as well as placental weight to birth weight ratio (PWR) in a large nationwide cohort accounting for multiple important confounders.

## PATIENTS AND METHODS

We conducted a nationwide cohort study including all Danish live births 1997-2012. Multiple pregnancies were excluded. CHD, PW, PWR, gestational age at birth and potential confounders were identified in national registries. In 30% of infants with CHD the diagnostic validity and the registration of genetic anomalies were validated in detail. The association between CHD, PW and PWR z-scores for gestational age was analyzed by multivariable linear regression unadjusted and adjusted for potential confounders including: infant sex, parental origin, maternal age, maternal BMI, maternal hypertension, maternal diabetes, maternal smoking, the presence of infant extracardiac malformations, congenital syndromes and year of birth.

## RESULTS

931,174 livebirths were included. CHD was present in 7,799 infants. Overall adjusted, CHD was

associated with a lower PW z-score -0.04 (95% CI -0.07; -0.02) and a larger PWR z-score, +0.08 (95% CI 0.06; 0.10). Three subtypes were associated with a reduction of both PW z-score and PWR z-score respectively: tetralogy of Fallot -0.44 (95% CI -0.57; -0.30) and -0.19 (95% CI -0.33; -0.05), double outlet right ventricle -0.54 (95% CI -0.92; -0.15) and -0.27 (95% CI -0.64; 0.10), major ventricular septal defects -0.39 (95% CI -0.51; -0.28) and -0.15 (95% CI -0.26; -0.03). Opposed to this, three subtypes of minor CHD were associated with larger PW z-score and PWR z-score respectively: pulmonary valve stenosis +0.11 (95% CI 0.01; 0.21) and +0.16 (95% CI 0.07; 0.25), patent ductus arteriosus +0.13 (95% CI 0.03; 0.23) and +0.13 (95% CI 0.03; 0.23), minor atrial septal defects +0.07 (95% CI 0.01; 0.13) and +0.18 (95% CI 0.12; 0.23).

## CONCLUSIONS

This is the first large study to show an association between placental anomalies, fetal CHD and growth in fetal CHD. We identified subtypes of CHD associated with both smaller PW and PWR whereas subgroups of minor CHD were related to larger PW as well as larger PWR. Future studies should address whether early placental anomalies may cause CHD, if placental anomalies and CHD share a common cause or whether CHD may cause placental anomalies per se.

# Structural and Functional Brain Imaging

## ABS 85

### RELATIONSHIP BETWEEN DIFFUSION TENSOR IMAGING AND FINE MOTOR FUNCTION IN YOUNG ADULTS BORN WITH VERY LOW BIRTH WEIGHT

K.A.I. Evensen<sup>1,2,3</sup>, I.M. Husby<sup>2</sup>, A. Olsen<sup>4,5</sup>, J. Skranes<sup>2,6</sup>, A.M. Brubakk<sup>2</sup>, A.K. Håberg<sup>7,8</sup>, L. Eikenes<sup>9</sup>

<sup>1</sup>Department of Public Health and General Practice, Norwegian University of Science and Technology, Trondheim, Norway

<sup>2</sup>Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway

<sup>3</sup>Department of Physiotherapy, Trondheim Municipality, Norway

<sup>4</sup>Department of Psychology, Norwegian University of Science and Technology, Trondheim, Norway

<sup>5</sup>Department of Physical Medicine and Rehabilitation, St. Olavs University Hospital, Trondheim, Norway

<sup>6</sup>Department of Pediatrics, Sørlandet Hospital, Arendal, Norway

<sup>7</sup>Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway

<sup>8</sup>Department of Diagnostic Imaging, MR-Center, St. Olavs University Hospital, Trondheim, Norway

<sup>9</sup>Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway

## INTRODUCTION

Being born preterm with very low birth weight (VLBW:  $\leq 1,500$  g) is associated with increased risk for developmental problems, including fine motor deficits. Studies have linked motor deficits to aberrant brain structures. However, few have examined several aspects of white matter microstructure in the same cohort in relation to motor function. Our objective was to examine associations between diffusion tensor imaging (DTI) measures and fine motor skills in a group of VLBW young adults compared with controls. We hypothesized that VLBW adults had lower fractional anisotropy (FA) and higher mean diffusivity (MD) than controls and that motor function was associated with white matter microstructure.

## PATIENTS AND METHODS

In a hospital-based follow-up study, 30 VLBW young adults without cerebral palsy (19 females, 11 males) and 31 term-born controls (17 females, 14 males) from a defined geographic region in Norway were examined with DTI and tests of fine motor function (Grooved Pegboard test: GP, Trail Making Test-5: TMT-5 and Turning pegs, Triangle and Drawing from the Movement Assessment Battery for Children-2: MABC-2) at mean age  $22.6 \pm 0.7$  years. Probabilistic tracking of the corticospinal tract (CST) and corpus callosum (CC) was performed. Volume, mean FA and mean MD were calculated for each tract. Between-group differences in DTI metrics and motor function were examined as well as associations between DTI metrics and motor function, adjusted for age, sex and laterality index.

## RESULTS

The VLBW group had higher MD and lower CST and CC volumes than the control group, but FA did not differ significantly. Performance on fine motor tasks was poorer in the VLBW group, however only significant for TMT-5 and MABC-2 Turning pegs.

In the VLBW group, poorer performance on TMT-5 and MABC-2 Triangle was associated with higher FA in both CST and CC ( $p \leq 0.01$ ), and time on GP and MABC-2 Turning pegs (non-dominant hand) was positively associated with FA in CST ( $p < 0.03$  and  $p = 0.002$ , respectively). There were no associations with MD or tract volumes and motor test performance in the VLBW group. In the control group no associations between motor function and FA, MD or volumes were found.

## CONCLUSIONS

Overall, VLBW young adults had smaller white matter tract volumes, increased MD values, and a different association between white matter structure and motor function, suggesting a different functional organization in VLBW individuals compared with controls. Our results may indicate that high FA is a sign of poorly developed motor tracts in the VLBW group.

## ABS 86

### INCIDENTAL FINDINGS ON ROUTINE MRI SCANS IN VLBW BABIES: SHOULD WE EXPECT THE UNEXPECTED?

M. Malova<sup>1</sup>, A. Parodi<sup>1</sup>, M. Severino<sup>2</sup>, G. Morana<sup>2</sup>, A. Rossi<sup>2</sup>, A. Sannia<sup>1</sup>, L.A. Ramenghi<sup>1</sup>

<sup>1</sup>Neonatal Intensive Care Unit, Istituto Giannina Gaslini, Genoa, Italy

<sup>2</sup>Pediatric Neuroradiology Unit, Istituto Giannina Gaslini, Genoa, Italy

## INTRODUCTION

Many NICU's have recently adopted the practice of performing routine brain MRI in Very Low Birth Weight (VLBW) babies at term corrected age, as it allows a better description of the severe acquired lesions, a better diagnosis of mild abnormalities, as well as evaluation of brain maturation. With the growth of the number of the studies that use MRI, grows the number of unexpected brain abnormalities that can be visualized, usually addressed to as incidental findings (IF). The aim of the study is to analyze and describe unexpected congenital abnormalities in this population of babies known to be at high risk just for acquired lesions.

## PATIENTS AND METHODS

We retrospectively reviewed a series of 206 consecutive MRI scans performed as a part of a follow-up program for all babies born in our Institute under 32 weeks and/or with birth weight under 1,500 g. Patients were screened at term corrected age from April 2012 to September 2014. All MRI

scans were performed on a 1.5 Tesla system using the “feed and wrap” technique.

All patients included in the study underwent serial cranial ultrasound (CUS) examination during the hospitalization. Scans were performed by an expert ultrasonographer according to our NICU routine protocol, which includes serial studies from birth until term-equivalent age. All ultrasound images were collected in an electronic database and retrospectively blindly reviewed to confirm the results.

## RESULTS

Among 206 VLBW patients, 110 (53.5%) presented prematurity-related acquired lesions at MRI, and in more than half of the cases the diagnosis was suspected already at CUS. On the other hand, 24 patients (11.6%) presented MRI findings that were considered incidental (not evidenced at ultrasound nor clinically suspected) (**Tab. 1**), and 14 of them (58%, or 6.8% of VLBW) required further intervention. In 2 patients with tuberous sclerosis and 7 with brain malformations genetic investigations and neurological follow-up were performed, and a patient with occult occipital meningocele was successfully operated. Three patients were directed to the endocrinologist suspecting ectopic neurohypophysis, and two of them started medical treatment. Two patients did MRI follow-up for cerebellar tonsillar ectopia, and one of them has developed Chiari I malformation with hydrocephalus, treated by neurosurgeons.

## CONCLUSIONS

Incidental findings can present a diagnostic and management challenge. Our study seems to suggest that these findings in VLBW are not rare, and in some cases it is possible to interfere with the prognosis, potentially ameliorating it. This new population of VLBW patients with many twin pregnancies, partly deriving from Assisted Reproductive Technologies may deserve a higher level of attention even in screening for subtle congenital abnormalities.

**Table 1 (ABS 86).** Acquired lesions vs. incidental findings on brain MRI in VLBW patients.

Acquired lesions	n (% of VLBW)	Incidental findings	n (% of VLBW)
Seen on CUS	69 (33.5%)	Irrelevant to the follow-up	10 (4.8%)
Not seen on CUS	41 (20%)	Relevant to the follow-up	14 (6.8%)
<b>Total</b>	<b>110 (53.5%)</b>	<b>Total</b>	<b>24 (11.6%)</b>

## ABS 87

### NEUROIMAGING IN PRESUMED PERINATAL ARTERIAL ISCHEMIC STROKE AND RECOGNITION OF SPECIFIC NEURORADIOLOGICAL PATTERN: A SINGLE CENTER EXPERIENCE

M. Bertamino, M. Severino, M. Di Rocco, A. Rossi, L. Ramenghi; Stroke Group\*

\*V. Capra, I. Ceccherini, M.E. Celle, C. Gandolfo, A.C. Molinari, A. Palmieri, M. Pavanello, P. Picco, S. Renna, A. Rossi, R. Ravazzolo

IRCCS G. Gaslini, Genoa, Italy

## INTRODUCTION

Stroke is increasingly recognized as an important perinatal disorder, with high morbidity and mortality rate. Until now, no neuroimaging pattern is recognized as specific for different etiology.

The aim of the study is to collect all patients with diagnosis of presumed perinatal arterial ischemic stroke admitted to Gaslini Hospital since 2005; to identify specific shared neuroradiological pattern in order to reveal imaging-etiology correlations and to select patient with idiopathic “Presumed Perinatal Ischemic Stroke” to be studied with next generation sequencing (NGS) for genes reported as cause of Arterial Ischemic Stroke (AIS).

## PATIENTS AND METHODS

We collected data from more than 2,000 MR and CT scan from 662 children (aged from 1 day and 18 years) with suspected diagnosis of AIS. Of those, 115 were classified as “Presumed Perinatal Ischemic Stroke” considering neuroradiological pattern and/or age of symptoms onset.

Clinical data were collected from electronic medical record systems of the Institute. The cerebrovascular imaging were analyzed by a neuroradiologist with more than 10 years of experience in pediatric neuroimaging.

Cerebrovascular imaging modalities included MRI or CT scan in 100% of cases completed by angiography MRI in 55.6%. 48.7% of patient performed also at least one follow-up MRI.

## RESULTS

Of the 662 case selected, 277 were excluded because of diagnosis of hypoxic-ischemic encephalopathy, and 270 were excluded with pediatric AIS or hypoxic contusive injury. Out of 115 patients with perinatal infarction, 81 had ischemic lesions, 22 had

porencephaly, 12 had both ischemic and hemorrhagic lesions. Left-sided infarction was present in 58% of children, right-sided in 24%, and bilateral in 18%. The majority of strokes occurred within the anterior circulations, involving the MCA territories in 89%. None had posterior circulation involvement. 23 patients performed first MRI within 7 days of life, 15 from day 8 and 30, the other from day 31 and 18 years. All data were compared relating to those categories. We could hypothesize a specific etiology in 32% cases: 19% had suspected pattern for *COL4A1* mutation, 8% for heart complaint, 3% had syndromic phenotype and 2% of stroke were related to infection.

### CONCLUSIONS

We could hypothesized a specific etiology, due to neuroradiological peculiar pattern, in a third of analyzed cases. This percentage drops to a quarter if we consider the range under the age of 7 days.

The use of NGS will allow us to better assess a possible correlation between neuroradiological subtypes and genotype. The diagnosis of single-gene disorders is required because may provide a benefit for genetic counseling and specific treatment.

### ABS 88

#### PUNCTATE WHITE MATTER LESIONS: CORRELATION TO GESTATIONAL AGE AND SWI APPEARANCE

M. Malova<sup>1</sup>, A. Parodi<sup>1</sup>, M. Severino<sup>2</sup>, G. Morana<sup>2</sup>, A. Sannia<sup>1</sup>, A. Rossi<sup>2</sup>, L.A. Ramenghi<sup>1</sup>

<sup>1</sup>Neonatal Intensive Care Unit, Istituto Giannina Gaslini, Genoa, Italy

<sup>2</sup>Pediatric Neuroradiology Unit, Istituto Giannina Gaslini, Genoa, Italy

### INTRODUCTION

Punctate white matter lesions (PWML) are often included in the spectrum of mild periventricular leukomalacia, due to potential similarities in pathogenesis, risk factors and gestational-age dependent vulnerability pattern. New data highlight heterogeneity of PWML, a possible explanation for the controversial prognostic meaning. Advanced MRI techniques, such as susceptibility weighted imaging (SWI), sensitive in detecting blood products, can be used to evidence subgroups of PWML. The aim of our study was to investigate differences between PWML with and without loss of signal on SWI in correlation with the gestational age in a group of consecutively admitted Very Low Birth Weight babies.

### PATIENTS AND METHODS

We have retrospectively reviewed MRI brain scans performed in 263 Very Low Birth Weight (VLBW) neonates admitted to our NICU, searching for PWML. All MRI scans were performed routinely at term equivalent age on a 1.5 Tesla system using “feed and wrap” technique.

PWML were then divided into two groups based on the evidence of blood degradation products as visualized by Susceptibility Weighted Imaging (SWI), and the prevalence of this finding was assessed in two gestational age (GA) groups (< 28 weeks and 28-32 weeks) according to previous hypothesis concerning the highest risk of developing periventricular leukomalacia.

### RESULTS

Out of 263 VLBW babies, 53 patients with PWML on MRI were selected. Among these, 17 (about 1/3) presented lesions visible on SWI as limited zones of lower signal in the periventricular zone, often following the distribution of deep medullary veins.

In the group < 28 week GA (78 babies), the incidence of punctate lesions was of 12.8%, with half of the lesions positive on SWI (**Tab. 1**). In 28-32 week group the total incidence of PWML was almost twice as high, although the share of SWI<sup>+</sup> abnormalities went down (28% of PWML).

Of interest, the incidence of SWI<sup>+</sup> PWML remained stable (6.4%) throughout the whole population of very premature children (< 32 weeks), highlighting a potential different vulnerability pattern when compared to SWI<sup>-</sup> lesions.

### CONCLUSIONS

About 1/3 of PWML present low SWI (SWI<sup>+</sup>) signal suggesting potential haemorrhagic-transudative process, as opposed to activation of ischemic-inflammatory pathway in SWI<sup>-</sup> lesions. The share of SWI<sup>+</sup> PWML goes down with the increase of GA, similarly to the risk of intraventricular haemorrhages. Punctate SWI<sup>+</sup> lesions could present

**Table 1 (ABS 88).** Punctate white matter lesions (PWML) in VLBW: gestational age and SWI appearance.

	GA (weeks)		Total
	< 28	28-32	
<b>Any PWML</b>	10	43	53
<b>% of population</b>	12.8%	23.2%	20%
<b>SWI<sup>+</sup> PWML</b>	5	12	17
<b>% of PWML</b>	50%	28%	32%
<b>% of population</b>	6.4%	6.4%	6.4%
<b>Population (VLBW neonates)</b>	78	185	263



a separate nosologic entity and should be studied further, especially in terms of follow-up.

## ABS 89

### PREDICTING SCHOOL-AGE COGNITIVE CAPACITIES FROM THE NEONATAL CONNECTOME IN PRETERM BORN CHILDREN

K. Keunen<sup>1,2</sup>, M.J.N.L. Benders<sup>1,2,3</sup>, A. Leemans<sup>4</sup>, I.C. van Haastert<sup>1</sup>, P.C. Fieret-Stam<sup>5</sup>, L.H. Scholtens<sup>2,6</sup>, M.A. Viergever<sup>5</sup>, R.S. Kahn<sup>2,6</sup>, F. Groenendaal<sup>1,2</sup>, L.S. de Vries<sup>1,2</sup>, M.P. van den Heuvel<sup>2,6</sup>

<sup>1</sup>Department of Neonatology, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>2</sup>Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>3</sup>Centre for the Developing Brain, King's College, London, UK

<sup>4</sup>Image Sciences Institute, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>5</sup>Department of Medical Psychology, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>6</sup>Department of Psychiatry, University Medical Center Utrecht, Utrecht, the Netherlands

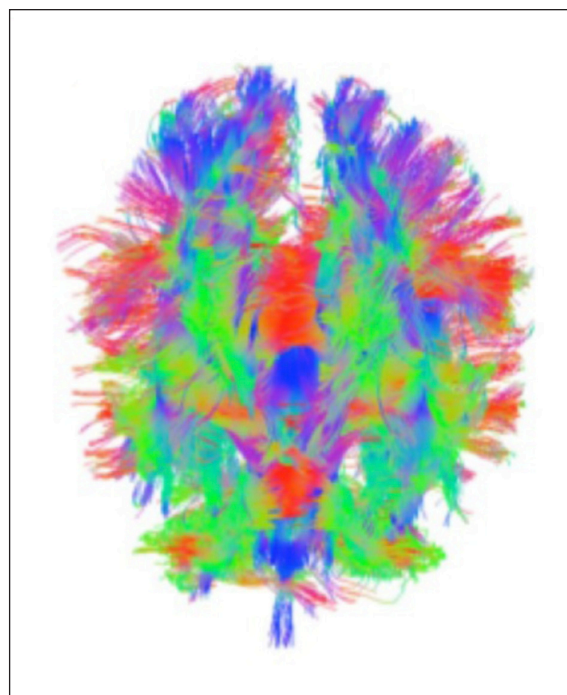
## INTRODUCTION

The organization of human brain wiring is known to be related to intellectual performance, with high performing individuals exhibiting more efficient connectivity patterns. However, when during connectome development the relationship between key features of the human brain network and cognitive functioning are established – and conversely how cognitive deficits are potentially related to early affected early connectome organization – remains unclear. Here, we examined the putative link between neonatal connectome organization (and disorganization) and cognition until early school age in a cohort of 57 preterm infants (mean gestational age  $28.1 \pm 1.8$  weeks, birth weight  $1,071 \pm 302$  grams).

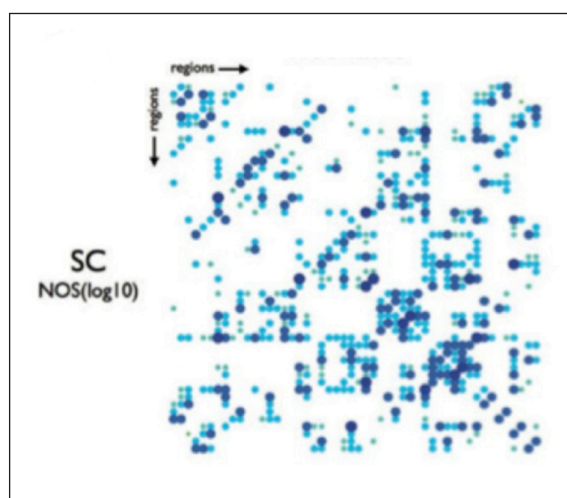
## PATIENTS AND METHODS

For each infant, a connectome map (Fig. 1A) was derived from diffusion weighted imaging data (3T MR, single shot EPI, 32 weighted diffusion scans ( $b = 800$  s/mm<sup>2</sup>) acquired at term equivalent age (TEA). Connectome maps were formed by selecting 56 cortical regions of interest and combining them with deterministic tractography to reconstruct the complete connectivity wiring between these regions. Of each individual connectome, its topological

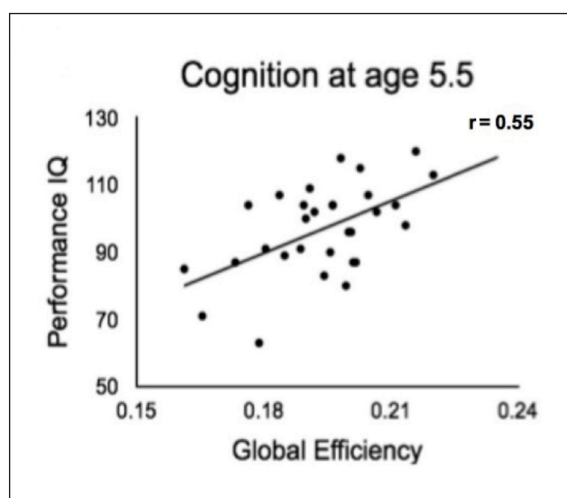
A.



B.



C.



**Figure 1 (ABS 89).** A. Connectome map. B. Topological organization by means of network analysis. C. Cognitive outcome at age 5.5 years.

organization was investigated by means of network analysis, with a particular focus on global efficiency (computed as the inverse of the average shortest path length in the network) (**Fig. 1B**). Next, cognitive outcome scores were obtained in early childhood (at age 2 years) using the BSITD-III and again at age 5.5 years in now school-going children, using the WPPSI-III NL.

## RESULTS

Neonatal network organization was found to be a significant predictor for childhood cognitive capacities. In particular global efficiency (i.e. the inverse of path length) of the neonatal network as a whole – reflecting how well the anatomical infrastructure can support global information integration at TEA – was observed to be significantly related to cognitive composite scores at age two ( $n = 57$ ,  $r = 0.26$ ,  $p = 0.04$ ), performance IQ (PIQ) ( $n = 31$ ,  $r = 0.55$ ,  $p = 0.002$ ), and full-scale IQ ( $n = 31$ ,  $r = 0.47$ ,  $p = 0.01$ ) at age 5.5 years (**Fig. 1C**). Clustering coefficient – measuring the level of segregation of the network into clusters specialized for local information processing – was also found to be associated with PIQ ( $r = 0.49$ ,  $p = 0.009$ ). These findings indicate that in particular higher levels of long-range communication capacity of the neonatal network correspond with better cognitive performance later in life.

## CONCLUSIONS

Our study provides evidence for a sustainable relationship between anatomical organization of the neonatal connectome and long-term cognitive capacities. Conversely, we hypothesize that alterations in neonatal network architecture may comprise a neurobiological substrate for cognitive deficits later in life. The neonatal connectome may thus offer a valuable imaging marker for early identification of infants at risk of these deficits.

## ABS 90

### CORPUS CALLOSUM-FASTIGIUM LENGTH; A NEW SONOGRAPHIC MARKER FOR PRETERM BRAIN GROWTH

J.A. Roelants<sup>1</sup>, I.V. Koning<sup>1,2</sup>, M.M.A. Raets<sup>1</sup>, S.P. Willemsen<sup>2,3</sup>, I.K.M. Reiss<sup>1</sup>, M.J. Vermeulen<sup>1</sup>, P. Govaerts<sup>1</sup>, J. Dudink<sup>1,4</sup>

<sup>1</sup>Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands

<sup>2</sup>Department of Obstetrics and Gynaecology, Erasmus MC, Rotterdam, the Netherlands

<sup>3</sup>Department of Biostatistics, Erasmus MC, Rotterdam, the Netherlands

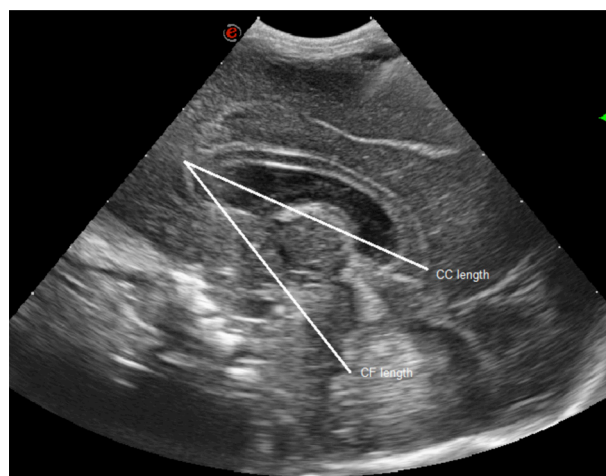
<sup>4</sup>Department of Radiology, Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands

## INTRODUCTION

Preterm infants are at risk for abnormal brain maturation. However, objective monitoring of brain growth in the neonatal period remains difficult. Cranial ultrasound (CUS) is a bedside neuroimaging method, however, still lacking reliable standardized measurements for evaluation of brain growth. We hypothesize that corpus callosum (CC) and corpus callosum-fastigium (CF) length (**Fig. 1**) are easy reproducible, quantitative markers for brain growth. In this study we evaluated the reproducibility of both measurements, created reference ranges and assessed whether peri- and postnatal variables influence CC and CF growth.

## PATIENTS AND METHODS

Between 2010-2012 all preterm infants, born before 29 weeks of gestation at the NICU of the Erasmus MC-Sophia Children's Hospital, were eligible for enrollment. We excluded infants with an unknown gestational age (GA), major congenital abnormalities, extensive IVH or posthaemorrhagic ventricular dilation. CUS was performed on day 0, 1, 2 and 7 after birth, and then weekly until discharge. One researcher acquired all scans and measurements (MR). Another independent researcher repeated measurements of 30 infants for assessment of inter- and intra-observer agreement (JR). This was evaluated by the method of Bland-Altman and interclass correlation coefficients (ICCs). Linear mixed



**Figure 1 (ABS 90).** Corpus callosum (CC) length and corpus callosum-fastigium (CF) length as quantitative markers for brain growth.

models were performed to evaluate associations between peri- and postnatal variables and brain measurements.

## RESULTS

Scans of 140 infants (median gestational age [range] 27<sup>2/7</sup> [23<sup>6/7</sup>-28<sup>6/7</sup>] weeks, mean birth weight [SD] 957 [267] gram) were analyzed. One to eight scans were performed per infant. ICCs showed good agreement for intra-observer agreement (ICCs CC: 0.911 [95%CI 0.806-0.959] and CF: 0.947 [95%CI 0.86-0.977]), and inter-observer agreement (ICCs CC: 0.886 [95%CI 0.682-0.953] and CF: 0.888 [95%CI 0.778-0.945]). Bland-Altman plots showed good agreement as well. Variables found to influence CC growth velocity were GA at measurement ( $\beta$  =

0.311 mm, standard error [SE] = 0.097,  $p < 0.01$ ) and birth weight (BW) SD score (SDS) ( $\beta$  = 0.075 mm, SE = 0.017,  $p < 0.01$ ). BW SDS ( $\beta$  = 0.049 mm, SE = 0.014,  $p < 0.01$ ) and female gender ( $\beta$  = -0.069 mm, SE = 0.029,  $p = 0.02$ ), showed to influence CF growth velocity.

## CONCLUSIONS

The reproducibility of both CC and CF length was high. This study, in which relatively healthy preterm infants were included, suggests that CC and CF length, as a measure for brain growth, is highly influenced by perinatal variables. More research is warranted to assess the clinical use and predictive value of these easy obtainable markers for neurodevelopmental outcome.